Egyptian Journal of Medical Microbiology Volume 31 / No.3 / July 2022 71-76 Online ISSN: 2537-0979

ORIGINAL ARTICLE

Sole Zinc Oxide and Titanium Dioxide nanoparticles antimicrobial activity versus their association with different antibiotics on Methicillin-Resistant *Staphylococcus aureus*

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Key words:
MRSA, Nanoparticles, ZnO NPs, TiO2 NPs

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ABSTRACT

Background: *Methicillin resistant Staphylococcus aureus (MRSA) is a major health issue and it is linked to high rates of morbidity and mortality. MRSA is a multidrug resistant organism therefore, several new antimicrobial agents against MRSA are urgently needed such as those based on nanoparticles (NPs).* ***Objectives****: Detection of the antimicrobial activity of ZnO and TiO2 NPS on MRSA if used alone then evaluating their effect when combined with different types of antibiotics.* ***Methodology****: This study was carried out on 150 pus samples collected from patients with infected wounds in different wards of Benha University hospitals. Identification of MRSA isolates was performed by chromogenic media and cefoxitin disc diffusion test. The antibacterial activity of ZnO and TiO2 NPs and the effect of their combination with antibiotics were tested by measuring their inhibition zones on MRSA isolates. The effect of NPs on MRSA isolates was traced by using electron microscope.* ***Results:*** *The prevalence of Staphylococcus aureus (S.aureus) among samples in the present study was 61(40.6%) and the percentage of MRSA among S. aureus isolates was 60.7%. This study detected a significant antibacterial activity of ZnO and TiO2 on MRSA isolates. There was a significant increase in the mean diameters of the inhibition zones of tested antibiotics on MRSA isolates when they were conjugated with ZnO and TiO2 NPs.* ***Conclusion:*** *ZnO and TiO2 NPs have a significant antibacterial activity on MRSA isolates. Our results support marked synergy between NPs (ZnO and TiO2) and antibiotics when both combined against MRSA isolates.*

INTRODUCTION

Staphylococcus aureus (*S. aureus)* is one of the most significant human pathogens that cause wound infections. It remains responsible for about one-third to one-half of all surgical wound infections [1].

S. aureus is a typical example for the rapid evolution of resistance to each new antibiotic which was put into clinical use. Methicillin was introduced in (1959) to treat infections caused by penicillin-resistant *S. aureus*. In (1961) there were reports some of *S. aureus* isolates that had acquired resistance to methicillin (MRSA) [2]. MRSA strains exhibit resistance to all β-lactam antibiotics through acquisition of the mobile staphylococcal cassette chromosome *mec* (SCC*mec*), which carries the antibiotic-resistant gene *mecA* that encodes expression of Penicillin Binding Protein2a (PBP2a) [3] which has low affinity to the beta lactam class of antibiotics [4]. MRSA is a multidrug resistant organism, being not only resistant to β-lactam antibiotics, but also resistant to other antimicrobial agents [5].

The development of new antimicrobial drugs based on nanoparticles (NPs) for the treatment of resistant pathogens such as MRSA may have many advantages compared with conventional antibiotics [6].Metal oxide nanoparticles such as zinc oxide(ZnO), titanium dioxide (TiO2) and silver oxide are attracting a great deal of attention because of their potential antimicrobial activity [7].

METHODOLOGY

This study was conducted in Medical Microbiology and Immunology Department, Faculty of medicine, Benha University, during the period from June 2020 to March 2021.It was carried out on 150 pus samples collected from patients having infected wounds in different wards of Benha University Hospitals.

This study was approved by the ethical committee of Faculty of medicine, Benha University.

Sample collection:

Infected wounds were sampled by sterile cotton tipped swabs rolled in all directions to obtain adequate amounts of pus**.**

Identification of *S. aureus* isolates:

The collected samples were transported rapidly to the laboratory for direct microscopic examination of Gram stained smears.

Samples were inoculated onto nutrient, and mannitol salt agar plates. The resultant colonies were identified as *S. aureus* by colonies morphology, Gram staining and biochemical characters of isolates [8].

Identification and antibiotic susceptibility of MRSA isolates:

Identification of MRSA isolates was performed by chromogenic media and by cefoxitin disc diffusion test**.** All MRSA isolates were tested for antibiotic susceptibility testing using the disk diffusion method which was carried out according to the methods of the Clinical and laboratory Standards Institute [9].

Sole using of NPs versus their association with antibiotics on MRSA:

ZnO and TiO_2 NPs were synthesized in Institute of Nanoscience and Nanotechnology, Kafr El Sheikh University. Broth microdilution method was used to determine the MIC of ZnO and TiO2 NPs [9]**.** The sterile discs were loaded with solution of the determined MIC for each NPs. Disk diffusion method was used to determine the inhibition zones the NPs on MRSA isolates.

Conjugated NPs with antibiotics (Azithromycin, clindamycin, linezolid, and vancomycin) were loaded on sterile discs and their inhibition zones were determined by disc diffusion method.

Tracing the effect of NPs on MRSA by using electron microscope

Electron Microscope was utilized for identification of morphological changes in MRSA isolates when treated with ZnO and TiO2 NPs.

RESULTS

Our study showed that out of 150 studied samples, 61(40.6%) were *S.aureus.* Out of 61 *S. aureus* isolates, 37 (60.7%) were MRSA as shown in Fig (1).

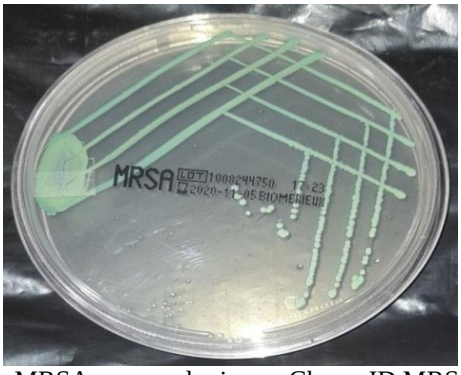
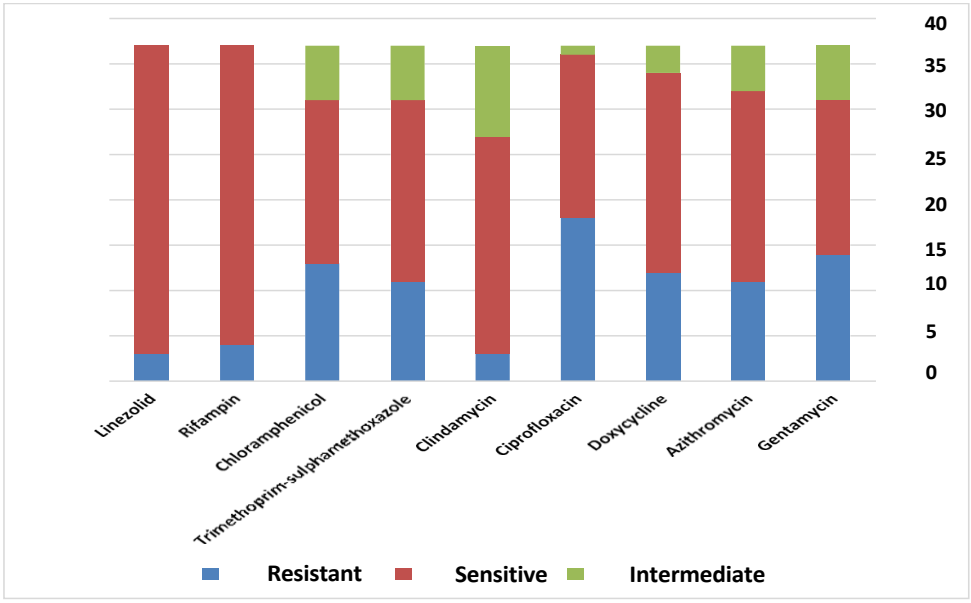


Fig. 1: MRSA green colonies on Chrom ID MRSA agar (BioMerieux, France)

The antimicrobial susceptibility of MRSA isolates showed that the highest antibiotic resistance rates were recorded for ciprofloxacin (48.6%), gentamycin (37.8%), and chloramphenicol (35.1%), while the lowest resistance rates were recorded for linezolid, clindamycin (8.1%) and rifampin (10.8%) respectively as shown in bar chart (1).



Bar chart .1: The antimicrobial susceptibility of MRSA isolates

The antibacterial effect of ZnO and TiO2 NPs on MRSA isolates was proved by measuring the inhibition zones around NPs discs (Fig 2). The mean diameters of inhibition zones of ZnO and TiO2 NPs on MRSA isolates were 8.16 mm and 6.08 mm respectively.

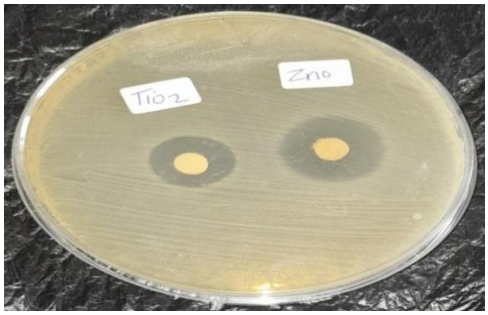


Fig.2. NPs susceptibility test for MRSA isolates

There was a high significant increase in the mean diameters of the inhibition zones of tested antibiotics on MRSA isolates when they were conjugated with ZnO and TiO2 NPs as shown in table1, table 2 and Fig3.

Table .1. The mean diameters of inhibition zones of antibiotics- ZnO NPs conjugates on MRSA isolates.

Antibiotic	Mean diameters of inhibition zones of Ab alone	Mean diameters of inhibition zones of Ab + ZnO	Paired *t- test*	*P -value*
Azithromycin	17.41±9.38	24.89±8.77	6.62	.000*
Clindamycin	22.19±6.09	29.68±7.15	9.24	.000*
Linezolid	26.57±6.58	33.00±7.46	7.71	.000*
Vancomycin	17.51±2.95	24.59±5.77	9.13	.000*

P value < 0.05 is significant; P value < 0.001 is highly significant

Table.2. The mean diameters of inhibition zones of antibiotics- TiO2

Antibiotic	Mean diameters of inhibition zones of Ab alone	Mean diameters of inhibition zones of Ab + TiO2	*Paired t-test*	*P -value*
Azithromycin	17.41±9.38	22.59±8.96	6.76	.000*
Clindamycin	22.19±6.09	27.81±8.36	7.01	.000*
Linezolid	26.57±6.58	31.41±6.01	7.02	.000*
Vancomycin	17.51±2.95	22.70±5.01	7.93	.000*

NPs conjugates on MRSA isolates.

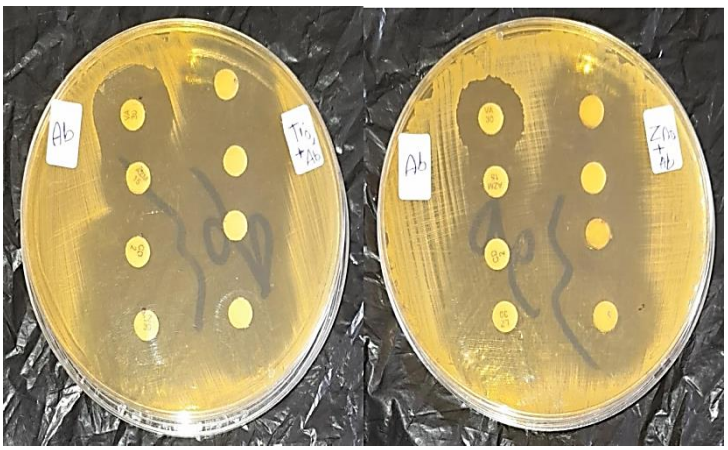


Fig. 3. Comparison of inhibition zones of antibiotics-NPs conjugates versus inhibition zones of antibiotics alone

The effect of NPs on MRSA by was traced by using electron microscope showing distortion in structure and arrangement of bacteria after treating with ZnO and TiO2 NPs as seen in Fig4.

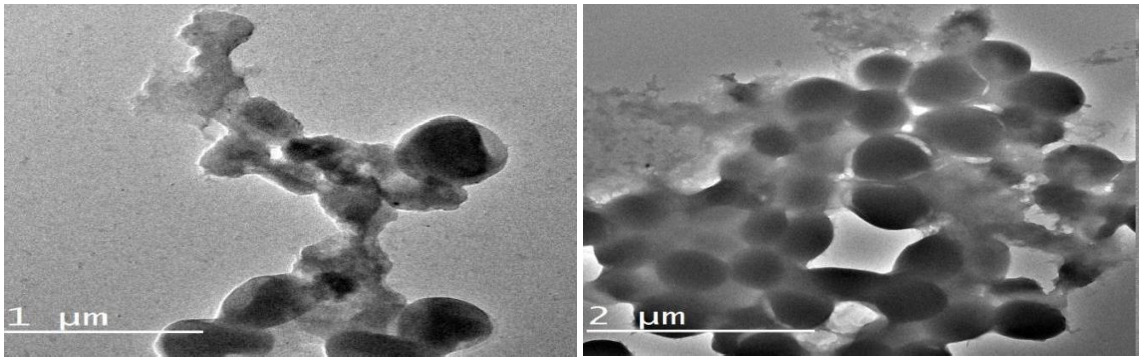


Fig.4. Distortion of MRSA structure and arrangement after treating with ZnO and TiO2 NPs

DISCUSSION

In this study *S.aureus* was isolated from 61(40.6%) of samples .This finding is in line with the study carried out by Chelkeba and Melaku [10] in which the percentage of *S.aureus* isolated from wound swabs was (36%). On the other hand, our finding is higher than a study conducted by Almeida et al [11] in which 20% of wounds were colonized by *S. aureus.* This dissimilarity can be attributed to different samples size taken in the other study.

The overall prevalence of MRSA in this study was 60.7%. This finding is similar to the results of Upreti et al [12] who reported that the percentage of isolated MRSA was 60.6%.In contrast to our result Abdulrahman and Nssaif [13] study revealed that the percentage of MRSA among *S. aureus* isolates was higher than this study (83%).

Concerning the antimicrobial resistance profile of MRSA isolates in the this study, results are consistent with results of Sharif et al [14] who reported low resistance rate of MRSA isolates to linezolid (7.14%).Likewise our findings agree Naimi et al [15] who reported that only 8.5% of MRSA strains were resistant to clindamycin. Our findings also agree with Ahmad et al study [16] that detected a low resistance rate to rifampin among MRSA isolates (5.3%).

Regarding our results of high resistance rates of MRSA isolates towards ciprofloxacin and gentamycin, they agree Kahsay et al [17] study which showed high level of resistance to gentamycin and Iram et al [18] who reported high resistance to ciprofloxacin. On the other hand, *Ahmad et al* study[16] showed that resistance rate to gentamicin and ciprofloxacin were lower than the percentage of resistance indicated by our study. The dissimilarity may be due to different clinical samples taken by Ahmad et al study beside wound swabs.

This study observed the evident antibacterial activity of ZnO and TiO2 NPs on MRSA isolates. These results are similar to a previous study which reported that average inhibition zone of ZnO and TiO2 NPs was 7 mm [13].Meanwhile another study [19] detected wider inhibition zones of ZnO and TiO2 on MRSA isolates (14.5 and 11 mm respectively).

Our results revealed marked synergy between ZnO and TiO2 NPs and tested antibiotics (azithromycin, clindamycin, linezolid and vancomycin) on MRSA isolates. This fining is consistent with Mubeen et al [20] which reported that ZnO and TiO2 NPs combinations with antibiotics especially glycopeptides (vancomycin) have shown promising enhancing effects in vitro.

The results of Iram et al [18]; Abdulrahman and Nssaif [13] and Abdelraheem et al [21] studies are in agreement with our findings that combining ZnO NPs with antibiotics (azithromycin, clindamycin and linezolid) has evident synergistic effect on MRSA isolates

Regarding the marked synergy between TiO2 NPs and antibiotics detected in this study; this is in consistent with Abdulrahman and Nssaif [13] and Shaikh et al [22] who reported that the antibacterial activities of tested antibiotics (including azithromycin and clindamycin and vancomycin) have been significantly increased in the presence of TiO2 NPs.

In this study, the effect of NPs on MRSA isolates was traced by using electron microscope showing distortion in structure and arrangement of bacteria and this finding is in agreement with Abd EL-Tawab et al [23] who reported significant changes in bacterial morphology when treated with NPs.

CONCLUSION

There is a high resistance rate of MRSA isolates towards many antibiotics and there is spread of resistance among critical drugs like linezolid .Our results support marked antibacterial activity of ZnO and TiO2 NPs against MRSA isolates. Our results support marked synergy between NPs (ZnO and TiO2) and tested antibiotics when both combined against MRSA isolates.

This manuscript has not been previously published and is not under consideration in the same or substantially similar form in any other reviewed media. I have contributed sufficiently to the project to be included as author. To the best of my knowledge, no conflict of interest, financial or others exist. All authors have participated in the concept and design, analysis, and interpretation of data, drafting and revising of the manuscript, and that they have approved the manuscript as submitted.

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