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# Nanomedical Applications of Titanium Dioxide Nanoparticles as Antibacterial Agent against Multi-Drug Resistant *Streptococcus Pneumoniae*

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## ABSTRACT

For four months (1st October 2019 to 31st January 2020), fifty sputum specimens collected from patients with suspected pneumonia in AL-Hilla General Teaching Hospital and Babylon Hospital for Pediatric and Gynecology in Babylon Province, Iraq. Only 10 isolates of *Streptococcus pneumoniae* were isolated and identified. Depending on the results of antibacterial susceptibility test with 16 antibiotics by disc diffusion method, *S. pneumoniae* isolates have multi-drug resistance. Full resistance(100%) against erythromycin, Penicillin G and Amoxicillin, full sensitivity(100%) for vancomycin and moxifloxacin, 90% for each Sulfamethoxazole/Trimethoprim and Tetracycline, 80% for each Azithromycin and Amoxicillin/Clavulanic Acid, 70% for Clindamycin, 50% for each Ceftriaxone and Chloramphenicol, 30% for Ciprofloxacin, and 20% for each Levofloxacin, Cefepime and Meropenem. By using agar-well diffusion method the antibacterial activity of Titanium Dioxide Nanoparticles (TiO2NPs) against *S. pneumoniae* was determined in three concentrations: 20, 30, 40 µg/ml. $TiO_2$NPs exhibit significant antibacterial efficacy in all concentrations while the biggest diameter of inhibition zone was measured in the higher concentration 40 µg/ml. MIC and MBC of $TiO_2$ Nano-particles were evaluated by using two-fold serial dilutions method, MIC ≥50 µg/ml while MBC ≥100 µg/ml.

**Keywords:** $TiO_2$ Nanoparticles, *Streptococcus pneumoniae,* antibacterial agent.

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## INTRODUCTION

The medical applications of nanotechnology included different fields such as medical applications of nanomaterial in nanomedical therapeutics, nanomedical diagnostics, nano electronic biosensors, antimicrobial nanomedicine and may be molecular nanotechnology at the future. It will hopefully allow the specialists to create more effective antibacterial therapeutics and more rapid and ultra-sensitive bacterial diagnostics and provide us further protection against pathogenic bacteria. recently, many novel nanocarriers have been provided in order to deliver the therapeutic agents by target-specific delivery. The studies reported that Nanoparticles have a broad-spectrum range of microbial agents more than antibiotics and can deal with various types of cells. Thus, nanoparticles have been candidate as an attractive alternative of antibiotics for the medical diagnosis and therapy [1-5]. On the other hand, many studies found that nanoparticles had a Biofilm inhibition activity in many bacterial species [6-8].

Recently, Antiviral nanomedicine is increasing concern as an important part of the nanomedicine, because the control of viruses (treatment or disinfection) is considered a big challenge in the health institutes. After the global spread of the COVID-19 pandemic, global health systems' suffering still in the face of this virus and the inability of common medicines to provide assistance. All attentions have been drawn to the nanoparticles and their potential role in both treatment and disinfection and many studies tried to the effectiveness of nanoparticles as diagnostic or antiviral tools against coronaviruses.

The possibilities of effectively using nanomaterials as vaccines and nanosensors in this field are also presented. many studies tried to prove the effectiveness of nanoparticles as diagnostic or antiviral tools against coronaviruses. Many studies tried to prove the potential effectively using nanomaterial such as Nano sensors, Nano based vaccine candidates and treatments for coronaviruses. The most important conclusions of these studies can be summarized, The Nano medicine field could be successfully applied for development of the most promising candidate drugs for coronaviruses as a treatment for the symptoms or as antiviral agents (concentration dependent responses). There are some advantages for using Nano sensors for diagnosing of coronaviruses, such as enhancing sensitivity with reducing the test time. in addition, nanoparticles may be used for simple, rapid and low-cost colorimetric test for coronavirus detection. on the other hand, depending to the previous studies on the other types of coronaviruses, the nano-based vaccine has been proven to induce a more potent immune response and need more studies to provide long-term immunization [ 9-13].

The dominance of multi-drug resistance in the pathogenic bacteria induced the bacteriologists to search about more suitable alternatives than antibiotics. Recently, the antibacterial nanomedicine nanoparticles represented the most attractive alternative as a new drug against bacteria. There is increasing interest of the antibacterial activity of nanoparticles of ZnO and $TiO_2$ against many species of pathogenic bacteria [14-17]. In order to get best results for using of nanoparticles in the battle against multi-drug resistant bacteria, It is taken into account that new classes, novel mechanism of action and different targets must be provided as compared as the traditional antibiotics. A review by Yacoby and Benhar [18] presented a number of nanomedicine-oriented applications of antiseptics, disinfectants and antibacterial therapeutics, by

summarized many studies described the antibacterial activity of nanoparticles against both Gram-positive and Gram-negative bacteria. Antibacterial activity of nanoparticles could serve as a marker for their potential general toxicity for the animals or humans. The evaluation of this potential toxicity, more complex applications (preclinical or clinical) must be done, in vivo, as actual therapeutics [19].

Among several candidates, $TiO_2$ stands out due to its potential for use in multifaceted applications. The clinical applications of titanium and it' s derivatives still account for a high percentage in the field of biomedicine and nano-medicine because of their biocompatible features[20]. Titanium dioxide nanoparticles($TiO_2$NPs) has wide using in the medical applications because of it' s specific properties such as the good stability(physical and chemical), low cost, non-toxicity, non-polluting behavior and high photo-catalytic activity under near ultrasound violate light illumination [21, 22]. Most importantly, the using of titanium dioxide as a nonspecific significant antimicrobial agent against a broad spectrum of pathogenic bacteria (even antibiotic resistant strains), fungi and viruses such as herpes simplex virus, influenza virus and zika viruse. The published studies have showed several mechanisms of antimicrobial action in the nanoparticles which make it the most appropriate promising candidate to overcome multidrug-resistant bacteria because the microorganisms are much less likely to develop resistance to metal nanoparticles compared with the common antibiotics [23-27]

*Streptococcus pneumoniae* is Gram-positive diplococci "pneumococci". It has many virulence factors helping of evasion from immune-system and invasion the lower respiratory tract. Although it is normal flora in upper human respiratory tract, pneumococci may be lethal pathogen by causing many severe infections, such as pneumonia, meningitis, septicemia, otitis media and bronchitis. Every year, during influenza season, pneumococci have the responsibility of higher mortality in the children worldwide because of it' s ability to damage the pulmonary cells causing pneumonia. in addition to it' s ability to cross the blood–brain barrier and infect the brain and spinal cords resulting in pneumococcal meningitis [28,29]

In Iraq, many molecular studies detected that most *S. pneumoniae* isolates have the genes responsible for the virulence factors such as *cspA* gene for capsule, *lytA* gene for autolysis, *ply* gene for pneumolysine, *PspA* for pneumococcal surface protein, *luxS* for luminescence S, ***α-Eno*** for α-enolase and *nanA* gene for neuroaminidase [30,31,32].

Although there is a vaccine, *S. pneumoniae* still represents a significant life-threatening pathogen in different ages worldwide. On the other hand, the emergence of penicillin resistant *S. pneumoniae* and dissemination in different regions and countries made it is a global significant challenge which hinders the control and treatment of pneumococcal infections in elderly and children [33,34]. In Asian countries, the studies have described the changing trends in antimicrobial resistance and serotype distribution of pneumococci and extremely high prevalence of multi-drug resistance [35,36,37].

## MATERIAL AND METHODS

**Specimens Collection, Bacterial Isolation and Identification**

Fifty sputum specimens were collected from patients with suspected pneumonia who visited AL-Hilla General Teaching Hospital and Babylon Hospital for Pediatric and Gynecology in Babylon Province, Iraq, during the period from 1st October 2019 to 31st January 2020. The age of patients was ranging from 5 to 65 years. The sputum specimens had been inoculated on specific culture media and incubated aerobically at 37°C for 24hr. The bacterial isolates were diagnosed depending on the colonial morphology, cellular microscopic examination and biochemical tests. Confirmation of identity of *S. pneumoniae* was performed with VITEK 2 system.

**Preparation of Bacterial Suspension**

Bacterial suspension was prepared by suspending 2-3 pure colonies of previously identified *S. pneumoniae* in 5ml of sterile brain heart infusion broth and incubated at 37ºC for 18h., it was standardized by gradually adding normal saline to compare their turbidity to McFarland standard.

**Preparation of $TiO_2$/ Dimethyl Sulfoxide Solutions (DMSO)**

Titanium dioxide nanoparticle stock solution was prepared by dissolving 10 mg of TiO2NPs in 10 ml dimethyl sulfoxide (DMSO) yielding stock solution of 1mg/ml concentration. 1 ml of the stock solution was diluted with 10 ml of DMSO giving a solution of 100 µg/ml concentration. Further dilution was done to prepare three concentrations included 40, 30, 20 µg/ml.

**Antibacterial Susceptibility test by Disc Diffusion Method**

This test was performed by using the disc diffusion method on chocolate agar with 16 antibiotics [38]. An inoculum from the bacterial suspension was streaking on a chocolate agar plate. The antibiotic discs were placed on the surface of the inoculated plate at evenly spaced intervals with flamed forceps. Incubation was usually overnight with optimal time of 18h at 37ºC. Antibiotic inhibition zone surrounded every disc was measured in millimeter and compared to standard criteria in CLSI [39]

**Screening of Antibacterial Activity of Titanium Dioxide against *S. pneumoniae***

$TiO_2$NPs have been gotten from laboratory of medical physics in faculty of pharmacy, university of Babylon where it has prepared according to Aysa [40] and examined by scanning electron microscopy (SEM).

Modified agar-well diffusion method was used to determine the antibacterial activity of TiO2NPs against *S. pneumoniae*. Chocolate agar plate was inoculated by streaking with bacterial suspension; each plate was performed in duplicates. Three concentrations of TiO2NPs solution (20, 30, 40) µg/ml were prepared previously. Four holes with a diameter of (6) mm were punched aseptically with a sterile corn borer (No. 6) on the inoculated plate. Approximately 20 µl of $TiO_2$NPs solution was introduced into each hole, one concentration for each hole. In addition, 20 µl of DMSO was introduced to the fourth hole as a negative control. One-hour pre-diffusion time was allowed at 4C°, then, plates were incubated at 37°C for 18h. The diameter of inhibition zone was measured in millimeter for the duplicates and the mean was taken

**Evaluation of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of $TiO_2$NPs against *S. pneumoniae*.**

MIC is the lowest concentration of antibacterial agent required to inhibit bacterial multiplication. In the present

study two-fold serial dilutions method was used in order to evaluate MIC of TiO2NPs. Six tubes of sterile brain heart infusion broth BHI (5ml) were prepared. As described in figure 1, stock solution of $TiO_2$NPs (200 µg/ml) and six dilutions (100, 50, 25, 12.5, 6.25, 3.125 µg/ml) were prepared with brain heart infusion broth. 0.1 ml of bacterial suspension of S. *pneumoniae* was added to each dilution. Finally, each tube containing 2.5 ml nanoparticle solution & 2.5 ml BHI &0.1 ml bacterial suspension. Control tube containing BHI was inoculated with the bacterial suspension without treatment with TiO2NPs solution. All tubes were incubated at 37-18h. Visually, MIC represents the first clear tube has no growth.

Minimum Bactericidal Concentration (MBC) is the least amount of antibiotic required to kill a bacterium. In the present study, MBC was determined by plating out the MIC tube and other clear tubes on chocolate agar plate as described in figure 2. The first tube has no growth on chocolate agar plate represents MBC because the bacteria has been killed firstly in this tube.

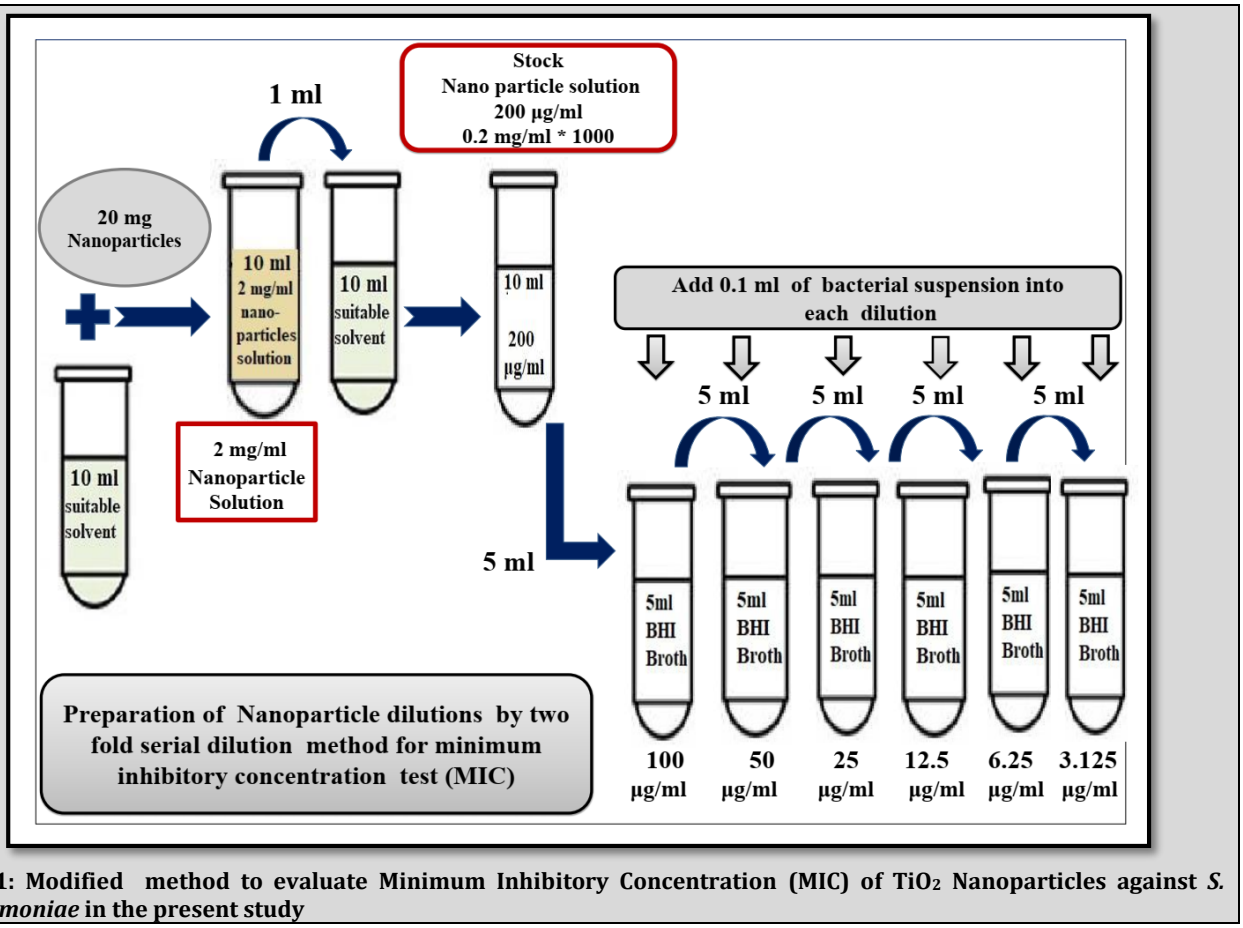


**Fig. 1: Modified method to evaluate Minimum Inhibitory Concentration (MIC) of $TiO_2$ Nanoparticles against *S. pneumoniae* in the present study**

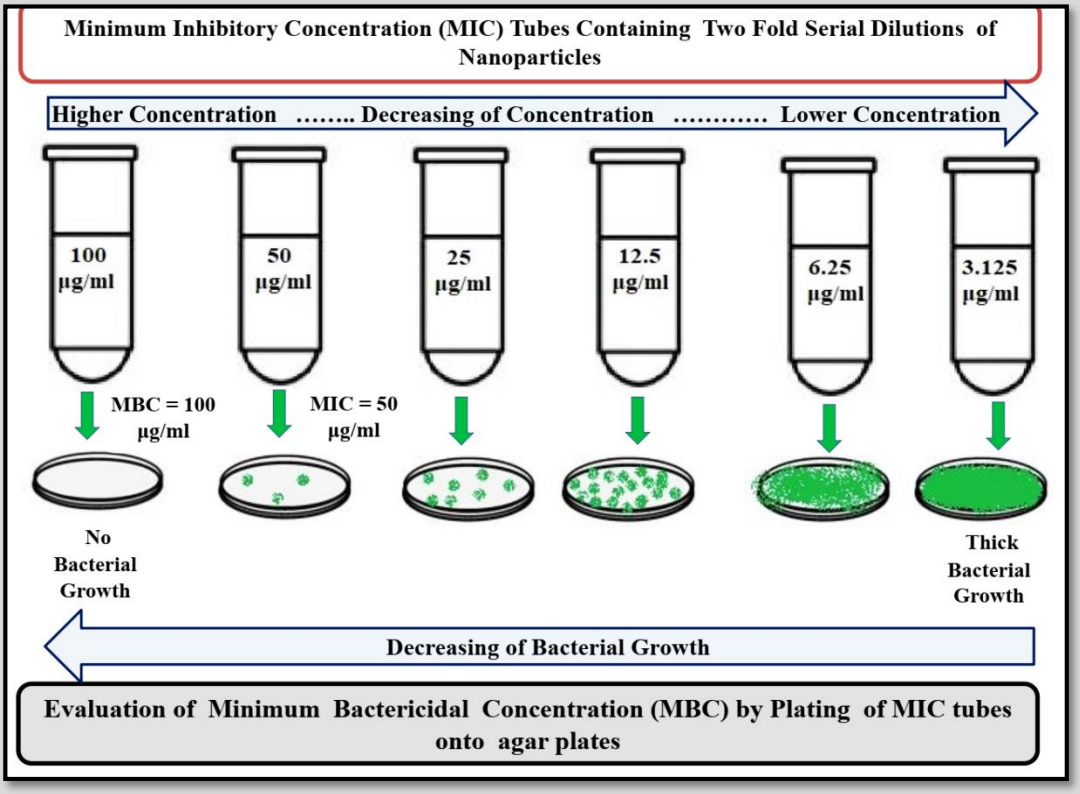


**Fig.2: Evaluation of Minimum Bactericidal Concentration (MBC) of $TiO_2$ Nanoparticles against *S. pneumoniae* in the present study.**

**Table 1: Diagnostic characteristics of *S. pneumoniae* in the present study**

| No. | The Test | The Result |
|---|---|---|
| 1 | Gram Stain | Gram-Positive Diplococci, Lancet Shaped |
| 2 | Cells Arrangement | Diplococci |
| 3 | Hemolysis | Alpha Hemolysis, Partial Hemolysis |
| 4 | Optochin Sensitivity | Sensitive |
| 5 | Bile Salt Solubility | Soluble |
| 6 | Autolysis | Positive |
| 7 | The Capsule | Positive |
| 8 | Catalase | Negative |

## RESULTS

### Specimens Collection, Bacterial Isolation and Identification

Among Fifty specimens of sputum, only 37 specimens have growth in the different culture media. Forty-six bacterial isolates were identified from these specimens (mixed infections). A total of 46 isolates were isolated and identified, included different species of gram positive and gram-negative bacteria caused pneumonia. Only 10(21.7%) isolates of *S. pneumoniae* were identified depending on the results of colonial morphology, cellular microscopic examination (table 1), optochin sensitivity (figure 3), biochemical tests and confirmative VITEK 2 system.

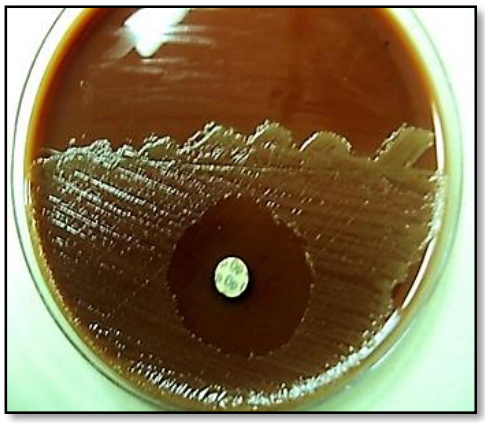

**Fig. 3: Optochin sensitive *S. pneumoniae* on chocolate agar in the present study.**

**Scanning Electron Microscopy (SEM) examination of $TiO_2$NPs**

Figure 4 portrays the SEM micrographs of TiO2 NPs. SEM micrographs uncovered group appearances with crystalline natures. Be that as it may, circular structures of size under 20 nm with unpredictable surface morphologies signified an expanded grain size because of the expansion in temperature prompting crystalline just as grain development. The stony appearance may have been because of the conglomeration of TiO2 NPs extending between 19-23 nm in size. these findings are comparable with the results of Dai *et al* [41].

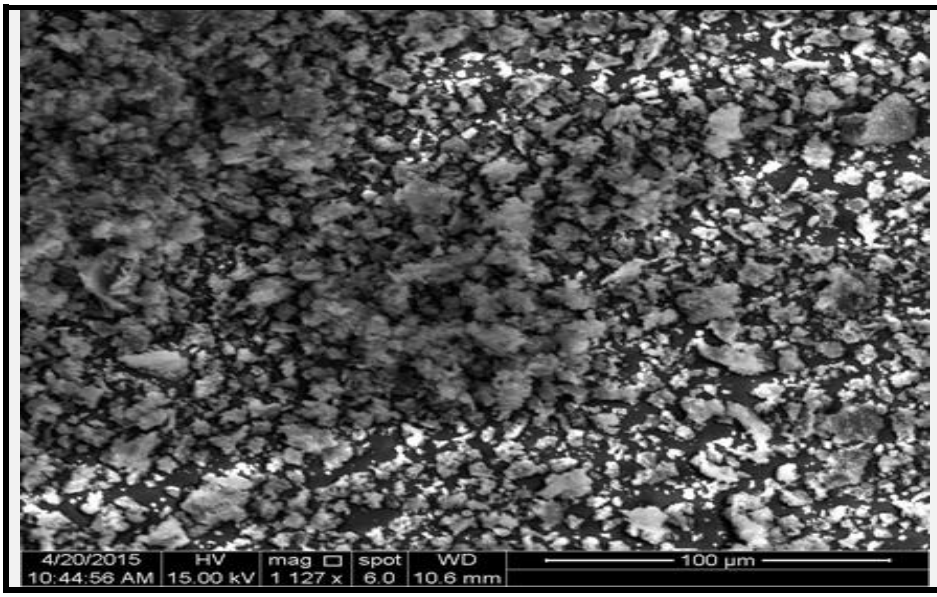


**Figure 4 portrays the Scanning Electron Microscopy (SEM) micrographs of TiO2 NPs in this study**

**Antibacterial Susceptibility test of *Streptococcus pneumoniae* by Disc Diffusion Method**

Disc diffusion test for antibacterial susceptibility of *S. pneumoniae* against sixteen antibiotic discs was performed in the present study. The results showed that *S. pneumoniae* has clear resistance against most common antibiotics, table 2. Depending on the diameter of inhibition zone surrounded the antibiotic discs, S. *pneumoniae* has a wide variety rang of resistance against different antibiotics. It has full resistance (100%) against erythromycin, Penicillin G and Amoxicillin. while full sensitivity (100%) was reported for vancomycin and moxifloxacin. The resistance percentage against other antibiotics was a variable as following: 90% for each Sulfamethoxazole/Trimethoprim and Tetracycline, 80% for each Azithromycin and Amoxicillin/Clavulanic Acid, 70% for Clindamycin, 50% for each Ceftriaxone and Chloramphenicol, 30% for Ciprofloxacin, and 20% for each Levofloxacin, Cefepime and Meropenem, Fig. 5.

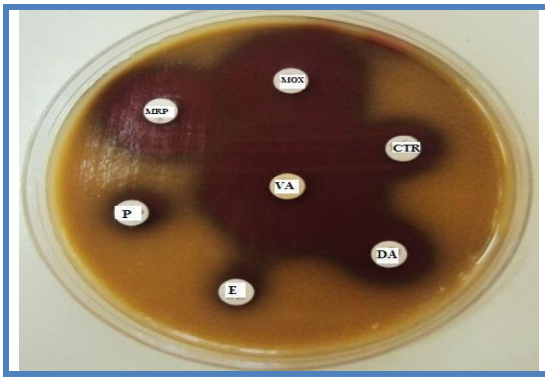


**Fig. 5: *S. pneumoniae* has full resistance against Erythromycin and full sensitivity against Vancomycin while the resistance against other antibiotics was variable between them.**

**Table 2: The percentage of antibiotic resistance of *S. pneumoniae* in the present study**

| No. | Antibiotic | Resistance % |
|---|---|---|
| 1. | Erythromycin(E) | 100 |
| 2. | Azithromycin (AZ) | 80 |
| 3. | Clindamycin (DA) | 70 |
| 4. | PenicillinG (P) | 100 |
| 5. | Amoxicillin (AMC) | 100 |
| 6. | Sulfamethoxazole / Trimethoprim (S/T) | 90 |
| 7. | Amoxicillin/Clavulanic Acid (AUG) | 80 |
| 8. | Ceftriaxone (CTR) | 50 |
| 9. | Cefepime (CPM) | 20 |
| 10. | Levofloxacin (LEV) | 20 |
| 11. | Ciprofloxacin (CIP) | 30 |
| 12. | Moxifloxacin (MOX) | 0 |
| 13. | Meropenem (MRP) | 20 |
| 14. | Tetracycline (TE) | 90 |
| 15. | Vancomycin (VA) | 0 |
| 16. | Chloramphenicol (C) | 50 |

**Screening of Antibacterial Activity of $TiO_2$ Nanoparticles against *S. pneumoniae*.**

Agar-well diffusion test was used to evaluate the antibacterial activity of $TiO_2$NPs against ten isolates of S. *pneumoniae* in the present study. As shown in table 3, $TiO_2$NPs exhibits significant antibacterial efficacy and the diameter of inhibition zones were influenced by the concentration of nanoparticle, Fig. 6.

**Evaluation of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of $TiO_2$ Nanoparticles against *S. pneumoniae*.**

In the present study, MIC and MBC of $TiO_2$ Nanoparticles were evaluated by using two-fold serial dilutions method with six dilutions 100, 50, 25, 12.5, 6.25, 3.125 µg/ml. As detailed in table 4 and fig.7, MIC ≥50 µg/ml for all bacterial isolates, While MBC ≥100 µg/ml.

**Table 3: The diameter of inhibition zones of titanium dioxide nanoparticles $TiO_2$NPs against *S. pneumoniae* in the present study**

| Conc. Of $TiO_2$NPs (µg/ml) | The diameter of inhibition zone in millimeter | | | | | | | | | | The Range of Diameter of Inhibition Zone |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Number of Isolate | | | | | | | | | | |
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | |
| 20 | 0 | 9 | 0 | 0 | 9 | 7 | 8 | 0 | 8 | 8 | 0-9 |
| 30 | 15 | 20 | 16 | 14 | 21 | 19 | 18 | 14 | 19 | 20 | 14-21 |
| 40 | 23 | 26 | 24 | 24 | 27 | 26 | 27 | 25 | 27 | 27 | 23-27 |
| Control | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

**Table 4: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of $TiO_2$NPs against *S. pneumoniae*.**

| Number of isolates | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| MIC | | | | | MIC ≥ 50 µg/ml | | | | | |
| MBC | MBC ≥ 100 µg/ml | | | | | | | | | |

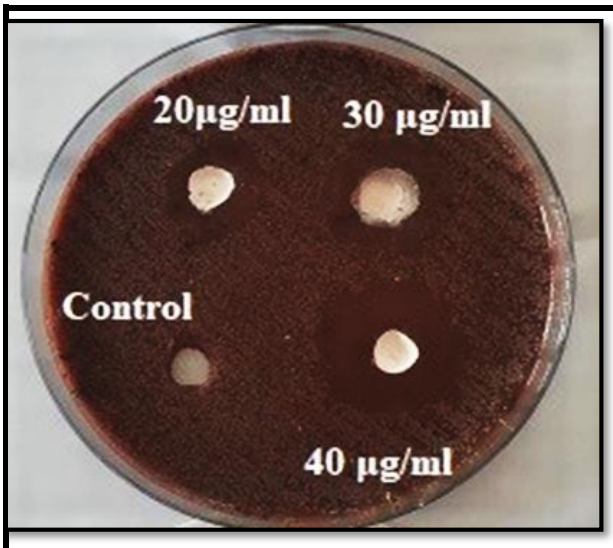


**Fig. 6: Antibacterial Activity of $TiO_2$NPs against *S. pneumoniae* was influenced by the concentration of nanoparticle solution.**

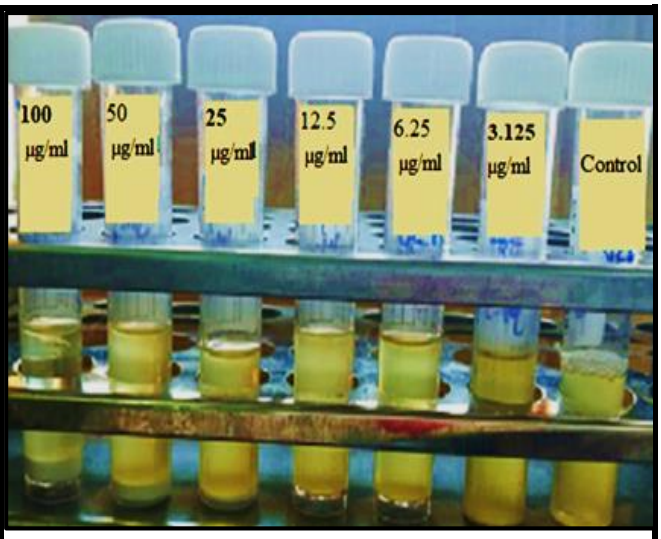


**Fig. 7: Six Dilutions 100, 50, 25, 12.5, 6.25, 3.125 µg/m were used to evaluate MIC and MBC for $TiO_2$NPs against *S pneumoniae*. MIC ≥50 µg/ml for all bacterial isolates, Whil MBC ≥100 µg/ml.**

## DISCUSSION

**Antibiotic Susceptibility test of S. *pneumoniae* by Disc Diffusion Method.**

In the present study the results showed that, *S. pneumoniae* isolates have full resistance 100% against each erythromycin, Penicillin G and Amoxicillin. While no resistance was observed against both vancomycin and moxifloxacin, as well as a variety percentage of resistance against other studied antibiotics were reported, table 2.

In Iraq, many publications studied the prevalence of antibiotic resistance in *S. pneumoniae* and their findings introduced an evidence for the truth that prevalence of multi-drug resistance depend on the geographical area. In Najaf province, full resistance in *S. pneumoniae* against erythromycin and high resistance against azithromycin (83.8%), clindamycin (83.8%), remarkable sensitive to Vancomycin (100%) and Imipenem (100%) were reported. In addition to the molecular detection of the genes responsible for the most common mechanisms of resistance to the macrolides in erythromycin resistant *S. pneumoniae* [40,41]. In Duhok city /Iraq, all isolates of *S. pneumoniae* have full sensitivity to vancomycin and penicillin and 50% to augmentin, but ≤ 41% to clindamycin, cephalothin, gentamicin, erythromycin, cefotaxime, and Co-trimoxazole [42,43].

On the other hand, our results are in disagreement with a study by Alfayate-Miguélez *et al* [44] who pointed out that, oral amoxicillin and intravenous penicillin or ampicillin represent excellent options for the treatment of *S. pneumoniae* isolated from nasopharyngeal samples and causes non-meningeal infections. Van Bambeke *et al* [45] suggested that, ketolides and fluoroquinolones could be considered for multi-drug resistant pneumococci treatment. The studies emphasized that more than 30% of *S. pneumoniae* are multi-drug resistant worldwide and suggested that the drug resistance genes of *S. pneumoniae* transfer by conjugative Transposon *Tn* which may play an important role in horizontal transfer and clonal dissemination of drug resistance genes in this bacteria [46,37]. In this study, we conclude that most isolates of S. *pneumoniae* developed multi-drug resistance which is often related to the availability of antibiotics out of hospitals which encourage self-medication.

**Screening of Antibacterial Activity of $TiO_2$NPs against *S. pneumoniae*.**

Recently, many types of nanoparticles were studied because of it' bacteriostatic and bactericidal efficacy which may be ascribable to the increasing surface area with decreasing size of nanoparticles. $TiO_2$ NPs which belong to the category of metallic nanoparticles have many applications as photosensitizing agents in the treatment of cancer as well as in photodynamic inactivation of antibiotic-resistant bacteria. The wide applications of $TiO_2$ and ZnO NPs are related to their low toxicity and high activity [47-52].

As detailed in table 3, $TiO_2$NPs exhibited significant antibacterial activity against *S. pneumoniae* in all concentrations.This finding is in accordance with that of other studies which showed that TiO2 NPs have inhibition ability for multidrug-resistant bacteria by specific

mechanisms such as releasing of positively charge ions leads to increase the cell wall permeability; structural deformation of DNA, ribosomes, and cellular enzymes; releasing of reactive oxygen and contributing in the degradation of biomolecules resulting in bacterial cell oxidization and finally bacterial cell death. This bactericidal characteristic of $TiO_2$ is primarily attributed to the oxidative stress present due to the production of reactive oxygen species (ROS) containing hydroxyl radicals and generation of hydrogen peroxide ($H_2O_2$) [53-57].

Notably, interesting with using TiO2NPs in food packaging and keeping was increased by researchers who showed that the packaging films coated by TiO2 NPs have ability to prevent the bacterial contamination on the food product surfaces by inhibition of biofilm formation by bacteria and therefore reducing the risks of food poisoning [58,59]. On the other hand, TiO2 NPs can reduce the biofilm formation in the glass surfaces in high rates [60].

In a study by Aysa [40] improved that $TiO_2$NPs Has antibacterial activity against gram positive and gram negative bacteria may be due to any energy stored inside the TiO2 to maintain low bacterial levels and reduce the risk of bacteria spreading around water reservoirs and surfaces that are often touched such as phones, keyboards and iPad covers. The mechanism of action of nanoparticles as antibacterial agents include some steps: first, the attachment of nanoparticle on the surface of the bacterial cell, then spreading through the cell wall followed by the adsorption on the cytoplasmic membrane and rupturing it which causes the leakage of the bacterial cytoplasm and finally cell death[61].

As shown in table 3, the diameter of inhibition zone and subsequently the antibacterial activity has increased with increasing of $TiO_2$NPs concentration, the biggest diameter of inhibition zone was measured in the highest concentration 40 µg/ml, Fig.7. this result is in agreement with other studies [62,63]. Hence, the appropriate concentration of nanoparticles play an important role and help to overcome drug resistance in the pathogenic bacteria [64]. This finding is comparable with Abdulazeem *et al.* [62] who concluded that ,in the suitable concentration$TiO_2$NPs have broad-spectrum antibacterial activity and can reduced the biofilm formation significantly.

**Evaluation of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of $TiO_2$ Nanoparticles against *S. pneumoniae*.**

As detailed in table 4, MIC ≥50 µg/ml for all bacterial isolates, While MBC ≥100 µg/ml, Fig. 8. These results are comparable with Abdulazeem *et al.* [62] who reported MIC of TiO2NPs ranged from 31.25 µg/ml to 125 µg/ml and the MBC ranged from 125 µg/ml to 500 µg/ml. A study by Lavaee *et al.* [47] has demonstrated that TiO2NPs alone have higher MIC and MBC than using them synergistically with other types of nanoparticles.

## CONCLUSION

$TiO_2$NPs exhibited significant antibacterial activity against *S. pneumoniae* which make it the most appropriate promising candidate to overcome multidrug-resistant bacteria because the bacteria are much less likely to develop resistance to nanoparticles compared with the common antibiotics.

**Ethical Approval and Consent to participate**

We gain the access permission from the authority of Al-Hillah General Teaching Hospital and Babylon Hospital for Pediatric and Gynecology to collect the samples together the verbal consent from patients and their relatives to take samples from them for scientific purpose with maintaining the safety of patient and respect his privacy. We offer our sincere thanks to all patients who agreed to participate in this study and made this work possible

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## CONFLICT OF INTEREST

The authors declare that they have no competing interests.

## REFERENCES

1. Weir E, Lawlor A, Whelan A, Regan F. The use of nanoparticles in anti-microbial materials and their characterization. Analyst. 2008;133(7): 835-45.
2. Kanwar J R, Mahidhara G, Kanwar R K. Recent advances in nanoneurology for drug delivery to the brain. Curr Nanosci. 2009; 5(4): 441-8.
3. Shi J, Votruba AR, Farokhzad OC, Langer R. Nanotechnology in Drug Delivery and Tissue Engineering: From Discovery to Applications. Nano Lett. 2020;10(9): 3223–3230. doi:10.1021/nl102184c.
4. Matthews, Liam, Rupinder K Kanwar, Shufeng Zhou, Vasu Punj and Jagat R. Kanwar. Applications of Nanomedicine in Antibacterial Medical Therapeutics and Diagnostics. The Open Tropical Medicine Journal. 2010; 3,1-9.1874-3153/10.
5. Akbar, A., Sadiq, M.B., Ali, I., Muhammad, N., Rehman, Z., Khan, M.N., Muhammad, J., Khan, S.A., Rehman, F.U., Anal, A.K. Synthesis and antimicrobial activity of zinc oxide nanoparticles against foodborne pathogens Salmonella typhimurium and Staphylococcus aureus. Biocatal. Agric. Biotechnol. 2019; 17, 36–42. https://doi.org/10.1016/j.bcab.2018.11.005.
6. Al-Shabib, N.A., Husain, F.M., Hassan, I., Khan, M.S., Ahmed, F., Qais, F.A., Oves, M,. Rahman, M., Khan, R.A., Khan, A., Hussain, A., Alhazza, I.M., Aman,S., Noor,S,. Ebaid, H., Al-Tamimi, J., Khan, J.M., Al-Ghadeer, A.R.M., Khan, M.K.A., Ahmad, I. Biofabrication of zinc oxide nanoparticle from ochradenus baccatus leaves: broad-spectrum antibiofilm activity, protein binding studies, and in vivo toxicity and stress studies.J. Nanomater. 2018; Vol.2018. https://doi.org/10.1155/2018/8612158.
7. Xu, S., Sun, T., Xu, Q., Duan, C., Dai, Y., Wang, L., Song, Q. Preparation and antibiofilm properties of zinc oxide/porous anodic alumina composite films. Nanoscale Res. Lett. 2018;13. https://doi.org/10.1186/s11671-018-2568-4.
8. Jasim, Nabaa A., Fadhl A. Al-Gasha'a, Mohammed F. Al-Marjani, Awas H. Al-Rahal, Hussein A. Abid, Nada A. Al-Kadhmi, Md Jakaria, Ahmed M. Rheima. ZnO nanoparticles inhibit growth and biofilm formation of vancomycin-resistant S. aureus (VRSA). Biocatalysis and Agricultural Biotechnology.2020; 29: 101745. https://doi.org/10.1016/j.bcab.2020.101745.
9. Ahmed SR, Kang SW, Oh S, Lee J, Neethirajan S. Chiral zirconium quantum dots: a new class of nanocrystals

for optical detection of coronavirus. Heliyon. 2018; 4(8), e00766.
10. Huang X, Li M, Xu Y, Jikang Z., Xia M., Xueying A., Lei S., Leilei G., Xue Sh., Junliang G., Jiao Ch., Yadong L., Heming W., Yu Z., Qing J., and Xinghai N. Novel gold nanorod-based HR1 peptide inhibitor for Middle East respiratory syndrome coronavirus.. ACS. Appl. Mater. Interfaces. 2019; 11(22), 19799–19807. https://doi.org/10.1021/acsami.9b04240.
11. Layqah LA, Eissa S. An electrochemical immunosensor for the corona virus associated with the Middle East respiratory syndrome using an array of gold nanoparticle-modified carbon electrodes. Microchim. Acta. 2019;186(4), 224.
12. Sekimukai H, Iwata-Yoshikawa N, Fukushi S, Hideki T, Michiyo K, Tadaki S, Hideki H, Kenichi N. *et al.* Gold nanoparticle-adjuvanted S protein induces a strong antigen-specific IgG response against severe acute respiratory syndrome-related coronavirus infection, but fails to induce protective antibodies and limiteosinophilic infiltration in lungs. *Microbiol. Immunol.* 2020; 64(1), 33–51. doi.org/10.1111/1348-0421.12754.
13. Nikaeen, Ghazal, Sepideh Abbaszadeh & Saeed Yousefinejad. Application of nanomaterials in treatment, anti-infection and detection of coronaviruses. Nanomedicine. 2020; 15(15), 1501–1512. Doi: 10.2217/nnm-2020-0117.
14. Al-Sa'ady AT. Antibacterial screening for five local medicinal plants against nosocomial pathogens: Klebsiella pneumoniae and Staphylococcus epidermidis. Eurasia J Biosci. 2020; 14: 553-559.
15. Huh, A.J. & Kwon, Y.J. Nanoantibiotics: a new paradigm for treating infectious diseases using nanomaterials in the antibiotic's resistant era. *J. Controlled Release*. 2011; 156, 128–145.
16. Ng, A.M.; Chan CM; Guo MY; Leung YH; Djurišić AB; Hu X; *et al.* Antibacterial and photocatalytic activity of TiO2 and ZnO nanomaterials in phosphate buffer and saline solution.Appl. Microbiol. Biotechnol. 2013;97(12): 5565–5573.
17. Al Sa'ady, Amal Talib. Detection of vancomycin resistance in multidrug-resistant Enterococcus faecalis isolated from burn infections. Drug Invention Today. 2019; 11(11):2984-2989.
18. Yacoby, Iftach & Itai Benhar. Antibacterial nanomedicine. Nanomedicine. 2008; 3(3): 329-341.https://doi.org/10.2217/17435889.3.3.329.
19. Melaiye, Abdulkareem; Zhaohui Sun; Khadijah Hindi; Amy Milsted; Daniel Ely; Darrell H. Reneker; Claire A. Tessier; and Wiley J. Youngs. Silver(I)–imidazole cyclophane gem–diol complexes encapsulated by electrospun Tecophilic nanofibers: formation of nanosilver particles and antimicrobial activity. J. Am. Chem. 2005; Soc.127,2285–2291.Crossref, Medline, CAS, Google Scholar
20. Habijan T; Haberland C; Meier H; Frenzel J; Wittsiepe J; Wuwer C; Greulich C; Schildhauer TA; and Köller M. The biocompatibility of dense and porous Nickel-Titanium produced by selective laser melting. Mater Sci Eng C Mater Biol Appl. 2013; ;33(1):419-26. doi: 10.1016/j.msec.2012.09.008. Epub 2012 Sep 23. pubmed.
21. Vero,N; S. Hribernik, P. Andreozzi, M. Sfiligoj-Smole. Homogeneous self-cleaning coatings on cellulose materials derived from TIP/TiO2 P25, Fibers Polym. 2009; 10:716–723.
22. Li,F.T.; Y.Zhao; Y.j. Hao; X.j. Wang; R.h. Liu; D.s. Zhao; D.m. Chen. N-doped P25$TiO_2$–amorphous $Al_2O_3$ composites: one-step solution combustion preparation and enhanced visible-light photocatalytic activity, J. Hazard. Mater. 2012; 239-240 :118–127.
23. Agarwal A; Weis TL; Schurr MJ; Faith NG; Czuprynski CJ; McAnulty JF; Murphy CJ; and Abbott NL. Surfaces modified withnanometer-thick silver-impregnated polymeric films that kill bacteria but support growth of mammalian cells. *Biomaterials*. 2010; 31(4):680-90. PubMed. DOI: 10.1016/j.biomaterials.2009.09.092.
24. Saravanan M; Vemu AK; and Barik SK. Rapid biosynthesis of silver nanoparticles from Bacillus megaterium (NCIM 2326) and their antibacterial activity on multi drug resistant clinical pathogens. Colloids Surf B Biointerfaces. 2011; 88(1):325-31. PubMed. doi: 10.1016/j.colsurfb.2011.07.009.
25. Aysa, Noor Hadi. Evaluating of mechanical properties of (silicone/arabic gum/fish hask) composites used as pressure garment prosthetics. Periodicals of Engineering and Natural Sciences. 2019a;7(3): 1202-1208. doi.org/10.21533/pen. v7i3.679.
26. Khezerlou A., Alizadeh-Sani M., Azizi-Lalabadi M., Ehsani A. Nanoparticles and their antimicrobial properties against pathogens including bacteria, fungi, parasites and viruses, Microb. Pathogens. 2018; Vol.123:123:505–526. doi.org/10.1016/j.micpath.2018.08.008.
27. Kumar, P; P. Mahajan, R. Kaur; S. Gautam. Nanotechnology and its challenges in the food sector: a review. Mater Today Chem. 2020; 17:100332. doi: 10.1016/j.mtchem.2020.100332.
28. Kadioglu, A., Weiser, J., Paton, J., Andrew, w. (2008) The role of Streptococcus pneumoniae virulence factors in host respiratory colonization and disease. Nat Rev Microbiol. 2008; 6, 288–301. doi:10.1038/nrmicro1871.
29. Henriques-Normark,Birgitta and Elaine I. Tuomanen. The Pneumococcus: Epidemiology,Microbiology, and Pathogenesis. Cold Spring Harb Perspect Med. 2013; 3: a010215. doi: 10.1101/cshperspect. a010215.
30. Motaweq, Zahraa Y.; Habeeb S. Naher; HawraaA.Ali.Al-Dahhan. Phenotypic and Genotypic Characterization of Some Virulence Factors in Streptococcus pneumoniae Isolated from Patients with LRTI in Najaf Province/Iraq. International Journal of Scientific & Engineering Research. 2015a;6(8):459-465.
31. Abdul-Lateef, Lamees Abdul-Razzaq; Safaa H. Alturaihy and Shaima A. Alabass. M. Al-Taai (2016) Molecular Characterization of Some Virulence Factors of Streptococcus pneumoniae Isolated from Children with Acute Otitis Media in Hilla, Iraq. *British Biotechnology Journal*. 2016;(3) .10 DOI: 10.9734/BBJ/2016/22033.

32. Al Hajem, H.M., Almazini, M.A., & Khudaier, B.Y. Investigation of the presence of some virulence factors of the *Strep. pneumoniae* isolates among patients in Basra Governora. *Iraqi Journal of Science*. 2018; 59(3A): 1205-1215. http://scbaghdad.edu.iq/eijs/index.php/eijs/article/view/450.
33. Al-Sa'ady, Amal Talib Atiyah and Habeeb S. Naher. Study of etiologic multi-drug resistant bacteria of neonatal sepsis in Al-Hilla city. American Journal of Biomedicin. 2016; 4(11). DOI: 10.18081/2333-5106/016-11/513-533.
34. Engholm, Ditte Høyer; Mogens Kilian; David S. Goodsell; Ebbe Sloth Andersen; and Rikke Schmidt Kjærgaard. A visual review of the human pathogen *S. pneumonia*. *FEMS Microbiology Reviews*. 2017; 41(6): 854–879. https://doi.org/10.1093/femsre/fux037.
35. Kim, SH; Song JH; Chung DR; Thamlikitkul V; Yang Y; Wang H; Lu M; So TM; *et al.* Changing trends in antimicrobial resistance and serotypes of *S. pneumoniae* isolates in Asian countries: an Asian Network for Surveillance of Resistant Pathogens (ANSORP) study. Antimicrob Agents Chemother. 2012; 56(3): 1418–26. PubMed. doi: 10.1128/AAC.05658-11. Epub 2012 Jan 9.
36. Song JH; Jung S; Ko KS; Kim NY; Son JS; Chang HH; Ki HK; Oh WS; *et al.* High prevalence of antimicrobial resistance among clinical *Streptococcus pneumoniae* isolates in Asia (an ANSORP study). Antimicrob Agents Chemother. 2004; 48(6): 2101–7. PubMed. DOI: 10.1128/AAC.48.6.2101-2107.2004.
37. Dakang Hu; Zheng Sun; Xinhua Luo; Shuangchun Liu; Lianhua Yu; *et al.* Drug Resistance Characteristics and Macrolide-Resistant Mechanisms of Streptococcus pneumoniae in Wenzhou City, China. Med Sci Monit. 2016; 22: 2731–2735. PubMed. doi: 10.12659/MSM.896766.
38. Bauer,A.W.; Kirby,W.M.; Sherris,J.C. and Turck,M. Antibiotic susceptibility testing by a standardized single disk method. *Amer. J. Clin. Pathol.*1966; 45: 493-496.
39. Clinical and Laboratory Standards Institute, CLSI. Performance standards for antimicrobial susceptibility testing. 29th ed. CLSI supplement M100. Wayne, Pa: Clinical and Laboratory Standards Institute. 2019; Vol.37(1).
40. Aysa, Noor Hadi. Synthesized of thermally isolated antibacterial (Silicone/TiO2) Nano-composite paint for reservoirs, Ann Trop Med & Pub Health. 2019b; 21: SP2048-19.
41. Dai, Shuxi; Yanqiang Wu; Toshio Sakai; Zuliang D; Hideki Sakai; Masahiko Abe. Preparation of Highly Crystalline TiO2Nanostructuresby Acid-assisted Hydrothermal Treatment of Hexagonal-structured Nanocrystalline Titania/Cetyl trimethyammonium Bromide Nanoskeleton. Nanoscale Res Lett. 2010; 5:1829–1835.DOI 10.1007/s11671-010-9720-0.
42. Motaweq, Zahraa Y.; Hawraa A. Ali. Al-Dahhan; Habeeb S. Naher. Detection of Macrolide Resistance Genes *Ermb* And *Mefa/E* in Iraq. World Journal of Pharmaceutical Research. 2015b; 4(8):252-265.
43. Motaweq, Zahraa Y.; Habeeb S. Naher. Antimicrobial susceptibility of Streptococcus pneumoniae isolates causing LRTI in Najaf, Iraq. Environ. Socio. -econ. Stud. 2017; 5(2):10-18. DOI: 10.1515/environ-2017-0007.
44. Alfayate-Miguélez S, Ruiz Gómez J, Sanchez-Solis de Querol M, Guerrero Gómez C, Cámara Simón M,Ortiz Romero MM, *et al.* Sensibilidad de *Streptococcus pneumoniae* en ninosportadores ˜ sanos en Murcia (Espana). ˜An Pediatr (Barc). 2015; 83:183-190.
45. Van Bambeke,F.; Reinert, R.R.; Appelbaum, P.C.; Paul M. Tulkens & Willy,E. Peetermans. Multidrug-Resistant *Strep. pneumoniae* Infections. Drugs. 2007; 67, 2355–2382. doi:10.2165/00003495-200767160-00005.
46. Jung B, Park SY, Lee YW, Lee J. Biological efficacy of Streptomyces sp. Strain BN1 against the cereal head blight pathogen Fusarium graminearum. Plant Pathol J. 2013; 29(1): 52–58. doi: 10.5423/PPJ.OA.07.2012.0113.
47. Lavaee F; Faez K; Faez K; Hadi N; Modaresi F. Antimicrobial and antibiofilm Activity of silver, titanium dioxide and iron nanoparticles. American Journal of Dentistry. 2016; 29(6):315-320. PMID: 29178718. PubMed.
48. Ziental, Daniel; Beata Czarczynska-Goslinska; Dariusz T. Mlynarczyk; Arleta Glowacka-Sobotta; Beata Stanisz; Tomasz Goslinski; Lukasz Sobotta. Titanium Dioxide Nanoparticles: Prospects and Applications in Medicine. Nanomaterials. 2020; 10, 387; doi:10.3390/nano10020387.
49. Kim SC; Lee DK. Preparation of $TiO_2$-coated hollow glass beads and their application to the control of algal growth in eutrophic water. Microchem. 2005; J;80(2):227-32. https://doi.org/10.1016/j.microc.2004.07.008.
50. Khalfa EF, 1Nafaee ZH, Abdullah FN, Bdair GS, Niema RM, 1 Salman HD, Hussein FH, Synthesis and Characterizations of Zinc Oxide Nanoparticles-Loaded Chloramphenicol for Antibacterial Applications, Ann Trop Med & Public Health; 22(IV): S383. DOI: http://doi.org/10.36295/ASRO.2019.221217.
51. Liqaa H. Abd, Riyam Abbas, Aseel M. Aljeboree, Firas H. Abdulrazzak, Falah H. Hussein, Ayad F. Alkaim, Role of Semiconductors (Zinc Oxide as a Model) for Removal of Pharmaceutical Tetracycline (TCs) from Aqueous Solutions in the Presence of Selective Light, International Journal of Recent Technology and Engineering (IJRTE) ISSN: 2277-3878, 8, (2S3) 2019, 1461-1463.
52. Haider A. Alwan, Mohammed A. Karam, Hayder O. Hashim And Falah H. Hussein, Synthesis and Antibacterial Activities of Silver Nanoparticles, Asian Journal of Chemistry; 31, (1) (2019), 56-60.
53. Koseki, H, Shiraishi K, Asahara T, Tsurumoto T, Shindo H, Baba K, *et al*. Photocatalytic bactericidal action of fluorescent light in a titanium dioxide particle mixture: *in vitro* study. Biomed Res. 2009; 30(3):189-92. Doi:10.2220/biomedres.30.189.
54. Fadel, Qasim; Mufeed Ewadh; Ilham Bnyan. Biological Balance Role of Oxidative Status for Some Bacterial Species. Biology Agriculture and Healthcare. 2013; 3(4).
55. Jayaseelan C, Rahuman AA, Roopan SM, Kirthi AV, Venkatesan J, Kim S, *et al*. Biological approach to synthesize TiO2 nanoparticles using *Aeromonas hydrophila* and its antibacterial activity. J Babylon Univ Pure Appl Sci. 2013; 25:599.

56. Moongraksathum, Benjawan; Jun-Ya Shang; Yu-Wen Chen. Photocatalytic Antibacterial Effectiveness of Cu-Doped TiO2 Thin Film Prepared via the Peroxo Sol-Gel Method. Catalysts. 2018; 8(9), 352; https://doi.org/10.3390/catal8090352.
57. Ikram, M; J. Hassan; A. Raza; A. Haider; S. Naz; A. Ul-Hamid; J. Haider; I. Shahzadi; U. Qamar; S. Alib. Photocatalytic and bactericidal properties and molecular docking analysis of TiO2 nanoparticles conjugated with Zr for environmental remediation. RSC Advances. 2020; Issue 50. doi.org/10.1039/D0RA05862A.
58. Chawenqkijwaich C, Hayata Y. Development of TiO2 powder-coated food packing film and its ability to inactivate *Escherichia coli in vitro* and in actual tests. Int J Food Microbiol. 2008; 123(3):288-92. doi: 10.1016/j.ijfoodmicro.2007.12.017.
59. Ibrahem KH, Salman JA, Ali FA. Effect of titanium nanoparticles biosynthesis by Laactobacilllus crispatus on urease, hemolysin and biofilm forming by some bacteria causing recurrent UTI in. Iraqi women. Eur Sci J. 2014; 10:324-38. https://doi.org/10.19044/esj.2014.v10n9p%25p.
60. Chorianopoulos NG, Tsoukleris DS, Panagou EZ, Falaras P, Nychas G. Use of titanium dioxide (TiO2) photocatalysts as alternative means for Listeria monocytogenes biofilm disinfection in food processing. Food Microbiol. 2010; 28(1):164-70. https://doi.org/10.1016/j.fm.2010.07.025.
61. Kenawy, E.-R., Worley, S.D., and Broughton, R. The Chemistry and Applications of Antimicrobial Polymers: A State-of-the-Art Review. *Biomacromolecules.*2007; *8*(5):1359–1384. https://doi.org/10.1021/bm061150q.
62. Abdulazeem, Lubna; BahaHamdi Hakim AL-Amiedi; Hadeel Alana Alrubaei; Yasir H. AL-Mawlah. Titanium dioxide nanoparticles as antibacterial agents against some pathogenic bacteria. Drug Invention Today. 2019; 12(5): 963-967.
63. Al Sa'ady, Amal Talib and Yasser Haider Al-Mawla. Comparison of Effects Antibiotics and Natural Honey and Extracts of Plants on Escherichia coli Growth Isolated from Different Pathogenic Cases. Journal of University of Babylon for Pure and Applied Sciences. 2019; 27(3):420-434.
64. Ramstedt M; Cheng N; Azzaroni O; Mossialos D; Mathieu HJ; and Huck WT. Synthesis and characterization of poly (3sulfopropylmethacrylate) brushes for potential antibacterial applications. *Langmuir*. 2007; 23(6):3314–3321. PubMed. DOI:10.1021/la062670.