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Research article

ZnONPs synthesis, characterization and activity against bacterial species isolated from chronic dentoalveolar abscess

Amr A. El-Waseif*[1], Samir M. Halawa[2], Mohamed O. Abdel Monem[3], Saif S. Al-Janabi[3], Mahmoud M. Amer[3]

[1]*Botany and Microbiology Department, Faculty of Science (Boys), Al-Azhar University, Cairo, Egypt.*
[2]*Oral and Maxillofacial Surgery in Banha, University Hospitals, Egypt.*
[3]*Botany and Microbiology Department, Faculty of Science, Banha University, Egypt.*

Key words: Dental Infection, *Staphylococcus,* Antibiotics, Zinc oxide nanoparticles, MIC.

***Corresponding Author: Amr A. El-Waseif,** Botany and Microbiology Department, Faculty of Science (Boys), Al-Azhar University, Cairo, Egypt.

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Abstract

Bacteria Causes Chronic Dentoalveolar Abscess usually treated using commercial antibiotics. Our research targeted the isolation of bacteria from 100 Chronic Dentoalveolar Abscess patients. Results of isolation part showed that 72 specimens recorded positive bacterial growth. Identification of bacterial isolates was performed using Grams stain and biochemical automated identification systems (VITEK). *Staphylococcus aureus, Kocuria rosea, Enterococcus casseliflavus, and Pseudomonas aeruginosa* were found in specimens taken from chronic dentoalveolar abscess in our patients. Molecular analysis 16S RNA used as confirmation test with the most potent pathogen and widely distribution 11M23 that act 92 % of total clinical isolates. Sequence data obtained that the sequence of 11M23 showed highest similarity (100%) to *Staphylococcus aureus*. Results of antibiotic sensitivity showed the multi-drug resistance bacterial species *Staphylococcus aureus* 11M23 obtained resist to Cefroxime, Oxacillin, Rifampin, Cefadroxil, and Amoxicillin. Synthesis of ZnONPs by chemical method performed and the resulted nanoparticles are characterized using TEM, UV-vis spectroscopy and Zeta potential. The antibacterial activity and minimum inhibitory concentration was carried out. ZnONPs can inhibit the growth of the *Staphylococcus aureus* in the obtained specimens at the minimum inhibitory concentration 3.4 mM.

Introduction

A wide scope of human irresistible maladies brought about by *Staphylococcus spp.* had critical harmfulness properties. They are not typically separated from the oral cavity and when it happens they are viewed as a piece of the transient microbiota. A few reasons were created for changing in oral microbiota. In immunocompromised individuals, these microorganisms may happen in a higher number. Patients with periodontal infection speak to possible reservoirs of these pioneering microorganisms in the oral cavity. They can likewise be a disease hotspot for different individuals [1]. In the oral cavity and in periodontal pockets of patients with unending periodontitis, separates recognize and confirm the connection between the nearness of *Staphylococcus spp.* in the oral cavity and periodontal pocket.

Since 1990, the significance of antibiotic resistance of oral microorganisms has expanded. The exact use of antibiotics in dentistry is a central point in its improvement; it speaks to 8 to 10 % of prescribed antibiotics. The molecular choice of antibiotics selection of endorsed is constrained. These remedies should focus on the germs causing the infection. These germs are enhanced and their commonness shifts starting with one investigation then onto the next [2].

For a long time, the antimicrobial prescriptions have been used to control or execute microorganisms. However, microbial protection from these medications has created on an extremely expansive scale after some time, extraordinarily diminishing their effectiveness and is a consistently developing issue [3]. An investigation says that the medication safe contaminations will murder an additional 10 million individuals per year around the world - more than at present bite the dust from disease by 2050 except if move is made. Therefore, a standout amongst the most encouraging methodologies for defeating microbial opposition is the utilization of nanoparticles. Nanotechnology gave the answer for prescription since it can discover materials in nanoscale distance across that have upgraded bioactivity. The principle purpose behind their significance is the expanded explicit surface territory of these nanoparticles in contrast with their volume, which empowers their communication with bio-organics present on the viable cell surface. One of the renowned nanoparticles is zinc

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oxide nanoparticles which is one of metal oxide nanoparticles. Zinc oxide is a polar inorganic compound. It shows up as a white powder, about insoluble in water with numerous applications, for example, antimicrobial, injury mending, UV sifting properties, high synergist and photochemical activity, because of its exceptional mix of intriguing properties, for example, non-danger, great electrical, optical and piezoelectric conduct, solidness in a hydrogen plasma air and low cost [3].

The point outlined in isolation and identification of infects bacteria causes chronic dentoalveolar abscess. Synthesis of ZnONPs by chemical method and they came about nanoparticles have portrayed using TEM, UV-vis spectroscopy and Zeta potential. Finally, the antibacterial action and minimum inhibitory concentration will determine. The potential outcome concluded in focus and recommended on the addition of Zn nanoform to dental tools and material protect from infection and reduced the resistance of microorganisms to antibiotics.

Materials & methods

Samples collection

Hundred clinical examples were gathered from a few therapeutic focuses and medical clinics in Egypt; amid 2018. Samples were gathered from oral tainted patients utilizing clean oral hole swabs (under the direction of a specialist). Transfer tests to sterile cylinders that contained 5.0 ml of Amies transport medium with aseptic safety measures, vortex blended for 1minute, to scatter the bacteria. A loopful of scattered examples was vaccinated on different media. All aerobic isolates incubated for 24-48h at 37°C.

The isolation medium mannitol salt agar medium is suggested for segregating pathogenic Staphylococci from clinical examples, beautifying agents, and microbial limit tests. This medium made out of 5 g/l Enzymatic process of casein, 5 g/l enzymatic process of creature tissue, 1 g/l hamburger extricate, 10 g/l D-manitol, 75 g/l sodium chloride, 0.025 g/l phenol red and 15 g/l agar. Suspend the element of the medium in one liter of refined water. pH changed in accordance with 7.4 ± 2.0 before sterilization.

Blood agar is a universally useful improved medium regularly used to develop picky life forms and to differentiate microscopic organisms dependent on their hemolytic properties. Additionally used for recovery of microorganisms as a rich medium. Blood agar base media are indicated in standard strategy methods for nourishment testing. It comprises of 10 g/l meat remove, 10 g/l peptone, 5 g/l sodium chloride and 15 g/l agar suspend the element of the medium in one liter of refined water. pH changed in accordance with 7.3± 0.2 before, a great many sterilizations, cool to 45-50°C, include 5 % v/v sterile defibrinated sheep blood to sterile media at that point blend overwhelmingly.

Morphological Identification

The morphological characteristics cell shape and cells game plans were exhibited by Gram's stain on mannitol salt agar medium [4]. This medium made out of 5 g/l Enzymatic process of casein, 5 g/l enzymatic digest of creature tissue, 1 g/l hamburger separate, 10 g/l D-manitol, 75 g/l sodium chloride, 0.025 g/l phenol red and 15 g/l agar. Suspend the element of the medium in one liter of refined water. pH acclimated to 7.4 ± 2.0 before sterilization.

Biochemical automated recognizable proof frameworks (VITEK)

The VITEK framework began during the 1970s as a computerized framework for (microbial distinguishing proof and antimicrobial powerlessness testing (AST), has advanced today into the VITEK 2 framework, which naturally performs the majority of the means required for ID and AST after an essential inoculum has been readied and standardized. This framework permits dynamic investigation by perusing each test each 15 min. The optical framework joins multichannel fluorimeter and photometer readings to record fluorescence, turbidity, and colorimetric signs [5].

Molecular identification

Chosen strain was analyzed infinitesimally for cell morphology and biochemical properties in previous think about. PCR was utilized to intensify the 16S ribosomal DNA quality of strain. The 16S ribosomal DNA sequence was dictated by direct sequencing. All out DNA was confined by utilizing Wizard genomic DNA purification unit (Promega, Madison, USA). Preliminaries utilized for PCR and DNA sequencing. Enhancement of DNA was performed in a Mastercycler individual warm cycler (Eppendorf). The successions acquired were then assembled in silico (Vector NTI) utilizing covering zones between the different arrangements to frame the coterminous sequence. Phylogenetic examination was acknowledged by an arrangement of grouping accord of the 16S ribosomal DNA genes gathered in a worldwide database (Gene bank). The outcomes were then communicated in level of homology between the submitted arrangement and the successions coming about because of the database [6].

Antibiotic disks

Ten different antibiotic disks were used in this study. These antibiotics plates are gathered with standard inhibition zone for chosen anti-microbial circles. A paper circles are dried and after that set over the agar surface plates freshly vaccinated with the test microorganisms in

Nutrient agar vehicle for bacterial strain. Supplement agar continues to be a broadly utilized universally useful mechanism for developing non demanding microorganisms. Whenever required, enrichments can be added to this medium. It comprises of 3 g/l meat separate; 5 g/p Peptone, 5 g/l NaCl and 20 g/l agar suspend the element of the medium in one liter of refined water. pH acclimated to 7.0 before disinfection. The petri-dishes were kept in a cooler for one hour to allow homogenous dispersion of the antimicrobial specialist before development of the test microorganisms and afterward plates were brooded at 37°C for 24 hours for bacteria.

Preparation of ZnONPs from zinc sulfate powder

Zinc oxide nanoparticles were set up as indicated by [7] by wet substance technique with some alteration, using zinc sulfate powder with sodium hydroxide as forerunners and starch as balancing out operator. Starch 0.1% was dissolved in 500 ml of refined water by utilizing microwave. At that point, 0.1 M of zinc sulfate was added to the above arrangement under consistent blending to totally dissolving the zinc sulfate. From that point onward, 0.2 M of sodium hydroxide arrangement was included drop by drop under consistent blending until bringing about a white arrangement. The reaction was permitted to continue for 2 hours after total expansion of sodium hydroxide at that point permitted to settle overnight. From that point onward, the supernatant arrangement was the disposed of cautiously and the white hasten was kept. The remaining white hasten was washed multiple times by utilizing refined water, centrifuged at 5000 rpm for 5 min. Finally, the white accelerate was dried at 50oC and kept for further use.

Characterization of zinc oxide nanoparticles

Transmission electron microscopy

This examination was attempted to know the size and state of Zinc oxide nanoparticles. The TEM picture was carried out utilizing: Electron test miniaturized scale analyzer JEOL – JXA 840A, Model Japan. Thin movies of the example were prepared on a covered copper lattice by simply putting a little measure of the example on the network. At that point the film on the TEM network was permitted to dry and the pictures of nanoparticles were taken.

Ultraviolet spectrum

The UV range investigation was completed utilizing: T80+UV/VIS Spectrometer, PG Instrument Ltd. Range: 190-1000 nm.

Zeta potential spectrum

The Z-NTA procedure permits the zeta capability of nanoparticles in watery suspension to be estimated on a particle-by-molecule premise. The modified zeta potential example chamber is fitted with platinum anodes, which allow a variable electric recorded to be connected to test of nanoparticles suspended in fluid solution.

Antibacterial activity of ZnONPs

Nano-ZnO was tried in vitro for their antimicrobial exercises against *Staphylococcus aureus* by the agar diffusion method. The *Staphylococcus aureus* was kept up on supplement agar media in Petri dishes with a inner width 9 cm to give thin agar plates after cementing of thickness 3.4-3.5 mm. After hardening, disks of ZnO nanoparticles were exchange to the supplement plat surface. The Petri dishes were brooded at 5-8°C for 1-2 h to permit good diffusion, temporary inhibition of test organism growth, transitory restraint of test life form development and afterward hatched for 24 h at 37°C. After incubation the diameter of inhibition zone (mm) was estimated [8].

Determination of minimum inhibitory concentration

MIC was estimated utilizing agar weakening tests. After immunization of target microscopic organisms on Nutrient agar media with different groupings of ZnO nanoparticles, the development rates of microbes were controlled by tallying colony forming unit (CFU) in each plate. The plates which demonstrate no development after 24 h brooding were chosen 0.1 ml of sterile refined water was added to these plates and exchanged to crisp medium which had no ZnO nanoparticles. The most minimal focus from which the microscopic organisms don't develop when exchanged to new medium is MIC the least fixation from which the provinces showed up over crisp medium [9].

Results & discussion

Isolation of pathogenic isolates

A total of 100 clinical samples were collected from oral infected patients, cultivated on selective media for isolating pathogenic isolates from these samples. After cultivation, the results obtained were evident it 72 from this samples contain pathogenic isolates while another samples do not contain bacterial growth therefore, excluded this samples (Figure 1).

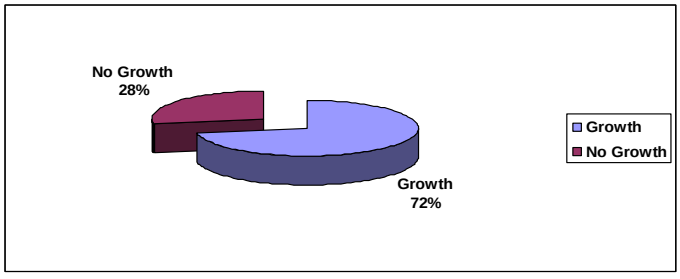


Figure 1. Infection percentage in 100 patients.

The samples contain pathogenic isolates distribution as the following, females 56, and males 16 from total infected 72 samples (Figure 2). This isolates were coded as samples number and (M) for male, (F) for female, while the final number for age. The infection in female was observed with 78% if compeer with male infected sampled.

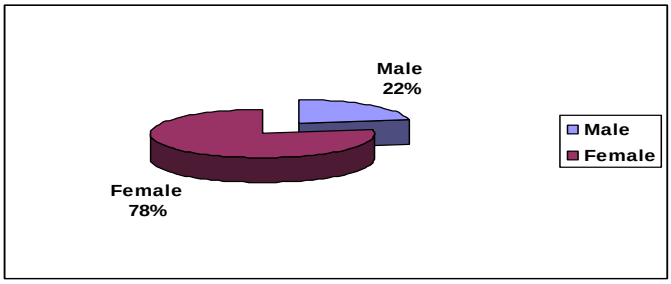


Figure 2. Distribution of infection percentage depending on gander.

The patients' age were ranged between 7 and 60 years old. Results obtained that 35 positive samples were recorded at age 21 to 40 years old. But 18, 19 positive samples were recorded at 7-20, 41-60 years respectively (Figure 3). The relation between source of samples, gender patient, age and diagnosis are recorded. The percentage of infection concentrated in age 20-40 years with 49 %.

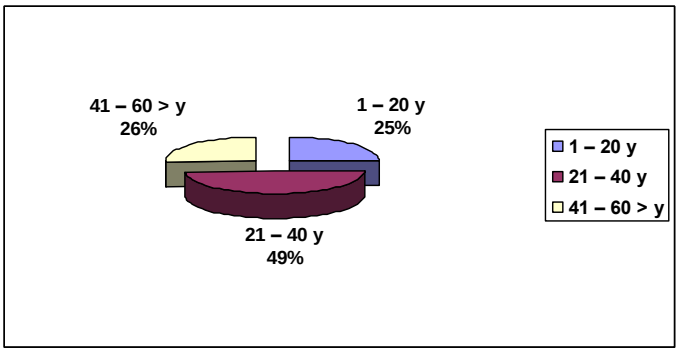


Figure 3. Effect of age on infection percentage distribution.

Morphological Identification

72 bacterial isolates were selected from collecting samples. The preliminary identification of pure bacterial isolates was based on cultural and morphological characters. Gram stain was used for morphological identification and classification of total 72 infected samples. The results obtained that bacterial isolates classified to 71 Gram-positive and one sample was Gram negative. Cell shape was obtained cocci, monococci, pairs and cluster in Gram positive samples and rods shape in Gram negative sample, but colony colors observed white, yellow, gold and pink.

Biochemical identification using Automated Identification Systems (VITEK)

Results indicated that one sample was Gram negative, rod shape, catalase positive and coagulase negative so selected for VITEK identification. Also, two samples were Gram positive, cocci, catalase negative and coagulase negative. The bacterial community structure and its dynamics were analyzed using morphological, and biochemical methods. Identification of isolates by VITK revealed that data recorded it's clear that *Staphylococcus aureus, , Kocuria rosea, Enterococcus casseliflavus,* and *Pseudomonas aeruginosa* respectively (Figure 4).

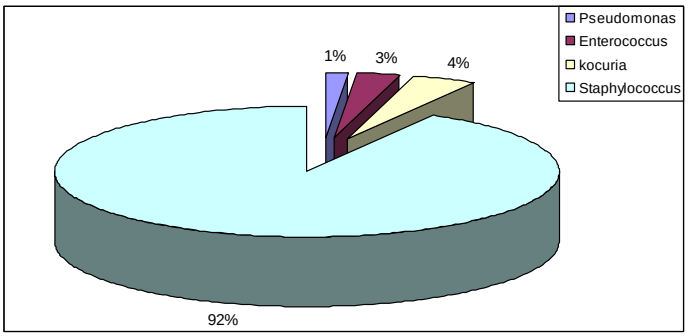


Figure 4. Distribution of bacterial pathogens in patient samples.

Molecular identification

Molecular analysis 16S RNA used as confirmation test with the multi-drug resistance bacterial species 11M23 that act 92 % of total clinical isolates. Sequence data of isolates were then analyzed by comparison with the genes of known bacteria available in the Gen Bank database, the obtained results revealed that the sequence of isolates showed highest similarity (100%) of isolate no 11M23 belonging to *Staphylococcus aureus* (Figure 5).

Sensitivity of the clinical bacterial isolates to antibiotics

The antibiotics sensitivity profile of bacterial detaches uncovered that the most antibiotics were bacterial secludes resistant Cefroxime and Oxacillin. Results demonstrated that the Gram negative Pseudomonas aeruginosa was sensitive to Rifampin, Vancomycin, Cefadroxil, and Amoxicillin individually. Results show likewise *Enterococcus casseliflavus* were exceedingly delicate to Vancomycin, Streptomycin and Rifampin individually. *Kocuria rosea* were recorded affectability to Vancomycin, Streptomycin and Rifampin individually. The significant pathogen *Staphylococcus aureus* indicates additionally delicate to Vancomycin, Streptomycin and Rifampin respectively.

The most powerful antibiotics agents against those detach were discovered Vancomycin, Streptomycin and Rifampin respectively. The outcomes demonstrate the example of multidrug obstruction bacterial disengages from oral figure 6.

In this manner, antibiotherapy at restricted range, for example, penicillin can be favored at first. An antibiotherapy is chosen as indicated by a few criteria: I) the causative pathogen operator ii) the powerlessness to

the picked particle and iii) the nonattendance of anti-microbial opposition of the pathogen. Therefore, several studies and global rules recommended the B-lactam group specifically the penicillin for its narrow spectrum, and a lesser danger of improvement of super infection [10].

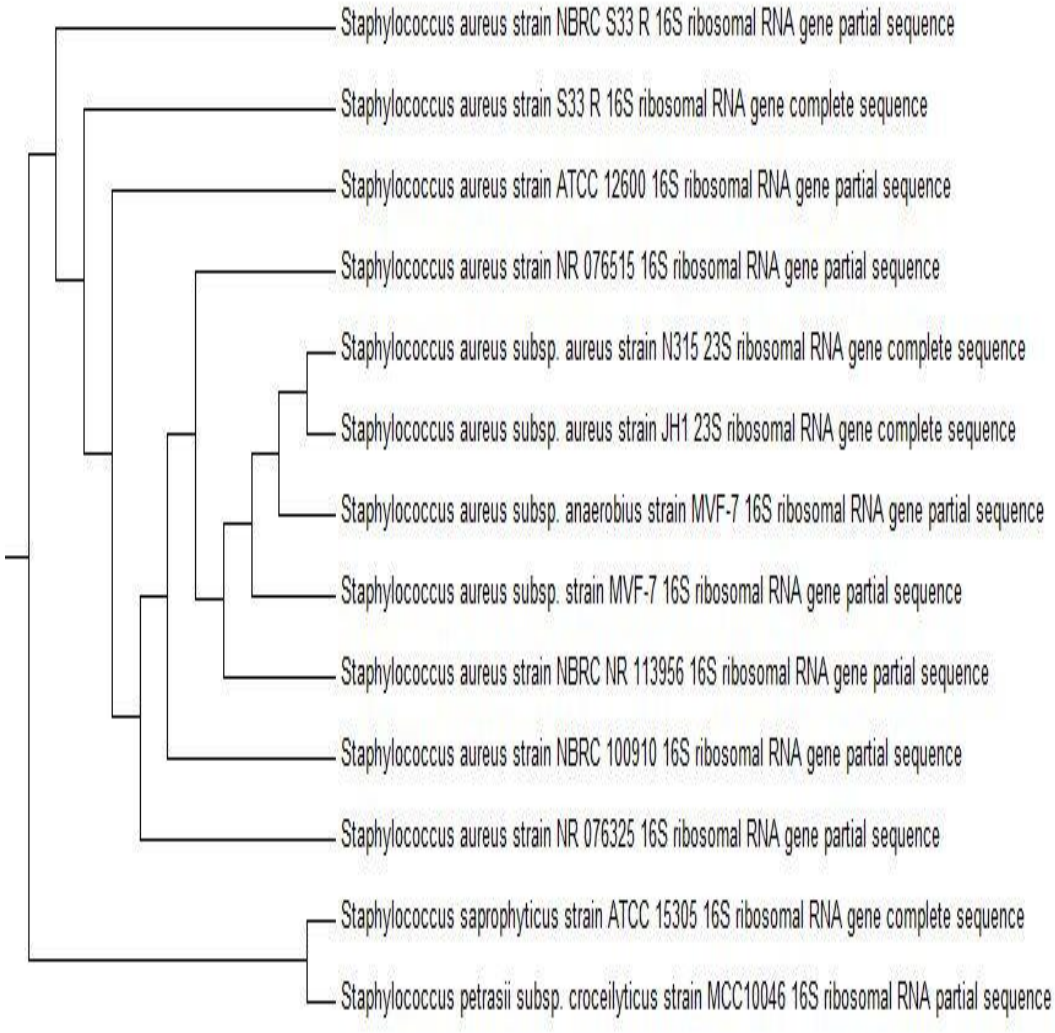


Figure 5. The phylogenetic tree of isolate 11M23 compared to other *Staphylococcus sp*.

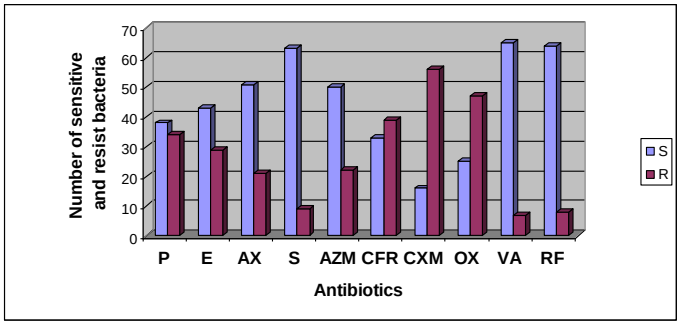


Figure 6. The relation between sensitive and resist bacteria to antibiotics (S: sensitive R: resist to antibiotic).

Synthesis and characterization of ZnONPs

By chemical method, ZnO nanoparticles performed successfully. During exposure of $ZnSO_4$ to sonication, reduction of zinc ions into zinc nanoparticles was monitored as a result of the color change to milky due to the Surface Plasmon Resonance phenomenon. Generally the ZnONPs have free electrons, which help in the formation of the Surface Plasmon Resonance absorption band. It happens due to the united vibration of the electrons of metal nanoparticles in resonance with light wave.

Transmission electron microscopy

The results of transmission electron microscope (TEM) for ZnO nanoparticles (ZnONPs) indicated that, the ZnONPs in the reaction mixture have a larger grain size, uniform shape and polycrystalline in nature. Also had a uniform spherical shape with varying sizes as observed in figure 7. Under magnification of 50 X the size of ZnONPs were ranging from 12.0 to 22.0 nm. Moreover, optic and clear rounded and oval shapes ZnONPs were detected; also, separated and conjugated nanoparticles were shown.

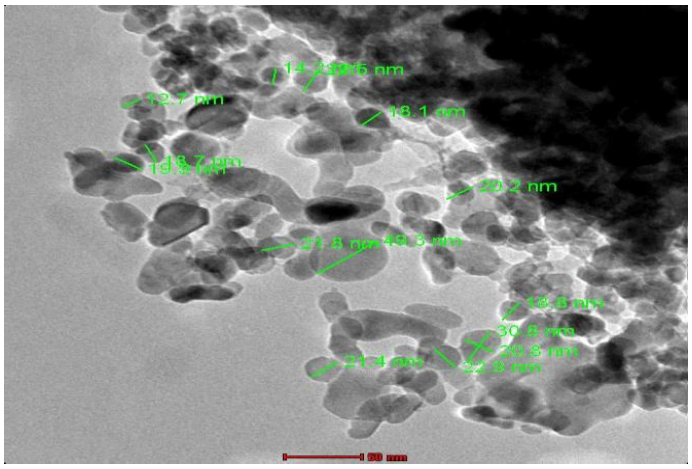


Figure 7. TEM images of zinc oxide nanoparticles processed from zinc sulfate powder.

Ultraviolet spectrum of ZnO nanoparticles

Figure 8 shows the UV-Vis absorption spectra of ZnO nanoparticles. The UV-Vis spectroscopic study shows the Plasmon resonance property, confirmed the reduction of metal ion and formation of nanoparticles with peak at 367 nm. The ZnO nanoparticles comprise distinguish color in colloidal solution due to its miniature dimension. The sharp bands of zinc colloids were observed at 367 nm.

On the surface of nanoparticles, the electron clouds are present which are able to oscillates and absorbs the electromagnetic radiation at a particular energy, energy corresponding to the photons of 367 nm. This technique was used to demonstrate the presence of zinc oxide nanoparticles which produced from zinc oxide powder. As illustrated in Figure 8, absorption peak of 367 nm was detected to confirm the presences of zinc oxide nanoparticles which processed from zinc sulfate powder.

Zeta potential of zinc oxide nanoparticles

Measurement of zeta potential was performed to study the stability of nanoparticles. Results of zeta values were found -2.73 mV at pH=7. The value of the zeta potential provides satisfactory evidence about their little tendency towards aggregation when its negative charges with a diameter of 22 nm.

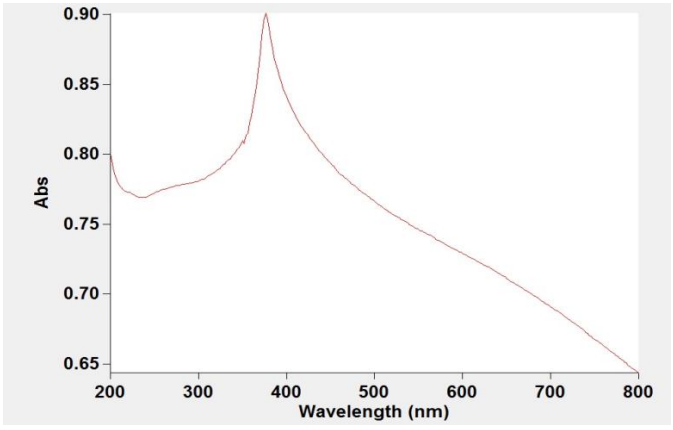


Figure 8. UV-spectrum of zinc oxide nanoparticles processed from zinc sulfate powder.

Antibacterial activity of zinc oxide nanoparticles

From the results obtained due to the antimicrobial activity of ZnO nanoparticles on the multi-drug resistance bacterial species *Staphylococcus aureus* 11M23 that showed resist to Cefroxime, Oxacillin, Rifampin, Cefadroxil, and Amoxicillin. it was interesting to note that as the concentration of nanoparticles increases, the zone of inhibition also increases and the highest inhibition zone was 28 mm at concentration 1 mg/mL.

Several researchers discussed the effect of zinc nanoparticles on the microorganisms and proved that, the activity of ZnO nanoparticles against the pathogen was due to a response of the surface of ZnO nanoparticles with water which led to formation of hoisted levels of receptive oxygen species, to be specific hydroxyl radicals and thus actuate as oxidative anxiety. Also, a presentation of microorganisms with ZnO nanoparticles results in an expanded cell disguise of the nanoparticles and microbial cell harm [11].

Among metal oxide powders, ZnO demonstrates very significant growth inhibition of a broad spectrum of bacteria. The suggested mechanism for the antibacterial activity of ZnO is based mainly on the catalysis of formation of the reactive oxygen species from water and oxygen that disrupt the integrity of the bacterial membrane, although additional mechanisms have also been suggested. Since the catalysis of radical formation occurs on the particle surface, particles with larger surface area demonstrate stronger antibacterial activity. Consequently as a result, the size of the ZnO particles decreases with increasing antibacterial activity.

Minimum inhibitory concentration

Number of colony forming unit (CFU) of *S. aureus* after overnight incubation at the presence of different concentrations of ZnO nanoparticles was carried out. The minimum concentration of ZnO nanoparticles which inhibited the growth of bacteria was 0.250 mg/ml. This is in agreement with previously published reports on the antibacterial properties of ZnO nanoparticles which showed that the minimum concentration at which the growth of *S. aureus* was inhibited was 3.4 mM [12].

MIC is defined as the lowest concentration of the anti microbial which inhibits the visible growth of a microorganism after the overnight incubation. But most are of research tools for the determination of the *in vitro* activity of new antimicrobials and the data from the study of it used to determine the MIC break points [13].

Conclusion

Staphylococcus aureus, Kocuria rosea, Enterococcus casseliflavus, and *Pseudomonas aeruginosa* respectively were infects bacteria causes chronic dentoalveolar abscess. Vancomycin the most potent antibiotic can be used against pathogens. ZnONPs by chemical method and characterized using TEM, UV-vis spectroscopy and Zeta

potential can inhibit the growth of the most distributed bacteria *Staphylococcus aureus* at the minimum inhibitory concentration 3.4 mM.

Acknowledgments

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