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Current Topics in Medicinal Chemistry, 2020, *20*, 1-14

RESEARCH ARTICLE

Antibacterial Assessment of Zinc Sulfide Nanoparticles against *Streptococcus pyogenes* and *Acinetobacter baumannii*

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Abstract: ***Background*:** Due to the appearance of resistant bacterial strains against the antimicrobial drugs and the reduced efficiency of these valuable resources, the health of a community and the economies of countries have been threatened. Objective: In this study, the antibacterial assessment of zinc sulfide nanoparticles (ZnS NPs) against Streptococcus pyogenes and Acinetobacter baumannii has been performed .

***Methods*:** ZnS NPs were synthesized through a co-precipitation method using polyvinylpyrrolidone (PVP), polyvinyl alcohol (PVA) and polyethylene glycol (PEG-4000). The size and morphology of the synthesized ZnS NPs were determined by a scanning electron microscope (SEM) and it was found that the average size of the applied NPs was about 70 nm. In order to evaluate the antibacterial effect of the synthesized ZnS NPs, various concentrations (50µg/mL, 100 µg/mL and 150 µg/mL) of ZnS NPs were prepared. Antibacterial assessments were performed through the disc diffusion method in Mueller Hinton Agar (MHA) culture medium and also the optical density (OD) method was performed by a UV-Vis spectrophotometer in Trypticase™ Soy Broth (TSB) medium. Then, in order to compare the antibacterial effects of the applied NPs, several commercial antibiotics including penicillin, amikacin, ceftazidime and primaxin were used.

***Results*:** The achieved results indicated that the antibacterial effects of ZnS NPs had a direct relation along with the concentrations and the concentration of 150 µg/mL showed the highest antibacterial effect in comparison with others. In addition, the ZnS NPs were more effective on Acinetobacter baumannii.

***Conclusion*:** The findings of this research suggest a novel approach against antibiotic resistance.

ARTICLE HISTORY

Received: November 26 2018
Revised: December 13 2019
Accepted: March 06 2020

DOI:
10.2174/1381612826666200406095246

Keywords: Zinc sulfide nanoparticles, Antibacterial effects, *Streptococcus pyogenes*, *Acinetobacter baumannii*, Antimicrobial resistance, Antibiotic resistance.

1. INTRODUCTION

Antibiotics react against bacteria in different ways to eliminate and reduce infection growth [1, 2]. The recognized platforms for such reactions include DNA destruction, cell wall degradation, directed effects on the cytoplasmic membrane, prevention of protein synthesis and anti-metabolites [3, 4]. The bacterial resistance occurs when mutations are enabled in the bacteria and they establish resistance against antibiotic drugs and new generations of them appear that cannot be fought against them easily [5]. The main cause of this type of drug resistance is self-treatment or excessive use of antibiotics [6]. This phenomenon endangers the entire human society [7]. Bacterial resistance against antibiotics is one of the biggest challenges in the modern age that threatens human health [7-9]. *Streptococcus pyogenes* is a gram-positive cocci; it is thought that about 700 million infections are caused by this bacteria annually and 650 thousands of these infections are severe and harmful (Fig. **1b**) [10, 11]. The mortality rate is about 25% for infections caused by this pathogen [12]. This bacterium creates important diseases in humans. Pharyngitis, impetigo, erysipelas, cellulitis and necrotizing fasciitis are some diseases caused by this pathogen [13, 14]. So far, penicillin and ampicillin are used as the most effective medical treatments against *Streptococcus pyogenes*. Compared with gram-positive bacteria, gram-negative bacteria are more resistant against antibiotics due to their impenetrable walls [15, 16]. Acinetobacter is a type of

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1568-0266/20 $65.00+.00

gram-negative bacteria that can be seen as bacilli or coccobacillus [17]. This bacterium is non-motile, oxidase negative and does not ferment sugars activity. This bacterium is resistant to most antibiotics, such as penicillin, but often sensitive to quinolones [18, 19]. *Acinetobacter baumannii* is an opportunistic pathogenic bacterium that leads to serious infections especially in hospitalized patients [17]. The mortality rate is high in blood infections for this bacterium and has been reported between 30-52% [20] (Fig. **1a**).

Additionally, *Acinetobacter baumannii* causes severe infections in susceptible individuals [22]. This bacterium is one of the main causes of nosocomial infections that can cause serious infections such as bacteremia, respiratory infections, urinary tract infections, pneumonia, endocarditis, wound and skin infections [22-24]. Many factors such as enzymes, exotoxins, siderophore and outer membrane proteins are involved in the pathogenesis of this bacterium. Clinical isolates of *Acinetobacter baumannii* have shown a significant increase in resistance against existing antibiotic treatments that have led to a challenge against this bacterium [18, 20, 25]. In recent decades, antibiotic resistance among strains of *Acinetobacter baumannii* has been increased alarmingly. Resistance against antibiotics has led to limited treatments against infections caused by this bacterium in patients [26]. Nanotechnology, as a new development in different scientific fields such as medical microbiology, has emerged [27-29]. In recent years, nanotechnology researchers have achieved great success in the production of new antimicrobial drugs against a variety of microorganisms and even for resistant strains to conventional drugs [30-38]. Nanomaterials have gained much attention due to their special features including their large surface to volume ratio and also high reaction activity that can have more captured bacteria [39, 40]. Properties of NPs depend on their size; the size control in NPs is performed for achieving different properties in various biomedical applications [41-51]. The reaction of materials in nanoscale is significantly increased and so far, numerous research works have been conducted to investigate the antibacterial properties of nanomaterials [52-56]. ZnS NPs are one of the important semiconductor materials from group II-VI with an energy bandgap of 3.7 ev between their full and empty electron balances [57, 58]. This material has a dielectric constant of about 2.35. ZnS NPs have very low toxicity and have recently been widely used due to their stable physical and optical properties and also high catalytic activity [48]. Moreover, the inexpensive synthesis and biocompatibility are other features of ZnS NPs. So far, several reports about the disadvantages of other usual nanomaterials such as silver [45, 51, 59], gold [45], copper [60], titanium [61, 62], zinc oxide [63] and magnesium oxide [64] have been reported. The major point that should be considered is the novelty, stability and highly controllable synthesis of ZnS NPs against other mentioned materials. In this study, the antibacterial property of ZnS NPs against *Streptococcus pyogenes* and *Acinetobacter baumannii* bacteria was studied in *in vitro* environment that suggests a promising approach to fight against antibiotic resistance.

2. MATERIALS AND METHOD

2.1. Materials

PVP, PVA, PEG-4000, zinc acetate dehydrate, sodium sulfide, fetal bovine serum (FBS), sodium pyruvate, sodium bicarbonate, trypsin- ethylenediaminetetraacetic acid (EDTA), trypan blue solution 0.4%, MTT (3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), phosphate-buffered saline (PBS), dimethyl sulfoxide (DMSO), distillated water (capacity 1500 mL), solid culture medium (MHA, 17.0 g/L), TSB double-strength bottled broth and nutrient broth medium (D (+)- glucose, 1 g/L) were purchased from Sigma-Aldrich (USA). DMEM/ F12 cell culture medium (Pan-Biotech, UK) and C6 rat glioma cell line (Bon Yakhteh cell bank, Iran) were the main materials and tools used for cellular assays. *Streptococcus pyogenes* (PTCC 1762) and *Acinetobacter baumannii* (PTCC 1797) bacteria were purchased from scientific and industrial research organization (Iran). Petri dishes, 96 well plate, antibiotics and other used materials and reagents were provided from standard sources with the highest available purity and quality.

2.2. Apparatus

Two incubators (Binder- Models BD 115 and C 170, China) were used to provide the optimum temperature for

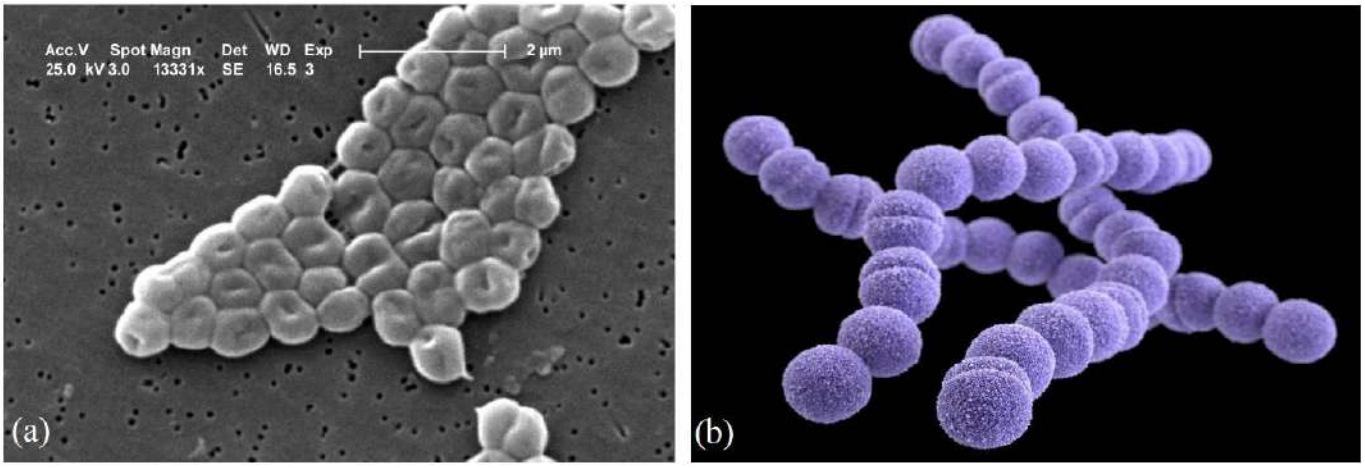

Fig. (1). Microscopic images, **a**): Acinetobacter baumannii; **b**): Streptococcus pyogenes [21]. *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

bacterial culture medium, bacterial growth condition and also for cell culture. In order to provide sterilization, an autoclave was used (Pars Mehr, Iran). A UV-Vis spectrophotometer (MACHEREY-NAGEL GmbH & Co. KG, Germany) was used to investigate and compare the antibacterial effects of ZnS NPs in TSB culture medium. The morphology of the synthesized ZnS NPs was investigated by an SEM microscope (SU3500 Premium VPSEM, Japan). Cell cultures' status was followed by an optical microscope (Olympus BH2, Olympus Corporation, Japan). A laboratory centrifuge (Sigma 2-16KHL – Germany) was used during some preparations. In order to investigate the cell viability and cytotoxicity of ZnS NPs on C6 rat glioma cell line, an enzyme-linked immunosorbent assay (ELISA) reader (BioTek, Model ELX800, USA) was applied for the analysis of related data.

2.3. Synthesis and Preparation of ZnS NPs

Here, ZnS NPs were synthesized in a composite form in the presence of PVA, PVP and PEG-4000 as the reducing and capping agents [48, 65-68]. The synthesis method was based on the co-precipitation in aqueous solutions (pH 8.4) [69]. Initially, a solution (50 mL) containing 0.25 g of all reducing agents was mixed with 50 mL of 0.1 M Zinc acetate dihydrate ($Zn(CH_3COO)_2 \cdot 2H_2O$) and stirred for half an hour by a magnetic heater stirrer (temperature: 50 ^oC) to get a homogeneous solution. Afterward, 50 mL of 0.1 M sodium sulfide was added slowly and continuously to the mentioned solution for 50 minutes (each minute 1000 µL). Then, a light color precipitate was created in the final solution that was lost with 2 minutes centrifugation (500 rpm). Finally, the solution was dried throughout the magnetic heater stirrer with 80^oC intended for 4 hours to obtain ZnS NPs as a white powder. The synthesis method used PVA, PVP and PEG-4000, simultaneously as catalytic degradation materials to improve the colloidal stability and reduce agglomeration [70]. These water-soluble polymers were applied due to their specific features, including NPs growth modifier, dispersant of ZnS molecules and reducing and capping agents. It should be considered that the solution in all steps of synthesizing was distilled water.

2.4. Preparation Bacterial Culture Medium

2.4.1. Preparation MHA

Firstly, according to the protocol, a specified amount of agar powder was dissolved in distilled water to reach pH 7.3. The temperature was 25 ^oC [71]. Then, this mixture was heated and a homogeneous solution was obtained. In the next step, the achieved solution was placed in an autoclave for sterilizing. Finally, the solution was poured in Petri dishes near the flame and they were incubated at 37 ^oC for 24 hours.

2.4.2. Preparation TSB Double Strength Bottled Broth

Firstly, according to the protocol, the specified amount of related powder was dissolved in distilled water to reach pH 7.3; the temperature was 25 ^oC. Then, this mixture was heated and a homogeneous solution was obtained [72]. In the next step, the achieved solution was poured in several culture tubes in an autoclave for sterilizing. Finally, the prepared culture tubes were stored in the refrigerator (4 ^oC) until use.

2.4.3. Preparation Nutrient Broth

Initially, according to the protocol, the specified amount of powder was dissolved in distilled water to reach pH 7.3; the temperature was 25 ^oC. Then, this mixture was heated and a homogeneous solution was obtained. In the next step, the achieved solution was poured into ten culture tubes in an autoclave for sterilizing. Finally, *Streptococcus pyogenes* and *Acinetobacter baumannii* bacteria were grown in these prepared tubes [73]. In this research, the nutrient broth culture medium was used as a bacterial growth medium and bacteria were cultured at 37 ^oC for 48 hours.

2.5. Methodology and Investigation of the Antibacterial Effects of ZnS NPs in MHA Culture Medium

In this method, the antibacterial effects of ZnS NPs were investigated against *Streptococcus pyogenes* and *Acinetobacter baumannii* bacteria in the MHA medium. Initially, the diluted microbial suspension was cultured on MHA plates with a sterile cotton swab. Various concentrations of ZnS NPs (50µg/mL, 100 µg/mL and 150 µg/mL) were used. Several cellulose acetate-based raw discs were used for the injection of NPs in sterile conditions. After drying, the related disc to each concentration of ZnS NPs was placed on a plate containing cultured bacteria. A culture medium with distilled water and without any ZnS NPs or antibiotic was used as the control group. In order to compare the antibacterial effects of ZnS NPs, some commercial antibiotics including penicillin, amikacin, ceftazidime and primaxin were used with a fixed concentration (200 µg/mL). The bacteria were grown at 37 ^oC for 24 hours. Then disc diffusion for various concentrations of ZnS NPs, control group and antibiotics was measured by a caliper. In addition, the bacterial colonies were counted.

2.6. Methodology and Investigation of Antibacterial Effects of ZnS NPs in TSB Culture Medium

In this method, the antibacterial effects of ZnS NPs were investigated against *Streptococcus pyogenes* and *Acinetobacter baumannii* bacteria in TSB medium. Initially, the diluted microbial suspension was cultured on TSB plates with a sterile loop. Various concentrations of ZnS NPs (50µg/mL, 100 µg/mL and 150 µg/mL) were applied. A culture medium without any ZnS NPs or antibiotic was used as the control group. In order to compare the antibacterial effects of ZnS NPs, some antibiotics, including penicillin, amikacin, ceftazidime and primaxin were used in a fixed concentration (200 µg/mL). The bacteria were grown in culture tubes at 37 ^oC for 48 hours. A UV-Vis spectrophotometer was used to measure the concentration of bacteria (OD: 600 nm).

2.7. Cell Culture for MTT Assay

In this study, a C6 rat glioma cell line was purchased from Bon Yakhteh cell bank (Tehran-Iran). Then, cell passages were applied in DMEM/ F12 medium (Pan-Biotech, UK) with 10% FBS, 100 mM sodium pyruvate, 1.5 g/ L sodium bicarbonate and penicillin-streptomycin antibiotic (1%) followed by incubation at 37 °C, 5% CO_2 and 90% relative humidity. Following this , when cells reached at least 70% cell growth, trypsin/ EDTA was used to detach adherent cells

from a culture surface; the detached cells were centrifuged at 1500 rpm for 5 min. The cell suspension was prepared in a 1 mL culture medium and the percentage of live cells in the suspension was determined by trypan blue through a hemocytometer slide and a light microscope. After evaluating to ensure that the cells were not infected, the cells with a percentage greater than 90% were used for the experiment.

2.8. Evaluation of Cell Viability and Cytotoxicity of ZnS NPs

In order to evaluate cell viability and cytotoxicity of various concentrations of ZnS NPs, the MTT colorimetric assay was followed. Initially, 5×10^5 cells/mL of C6 rat glioma cells were prepared and seeded into a 96 well plate; then, cells were settled to the bottom of each well within 24 hours and after this time, the supernatant was removed from each well. In the next step, cells were divided into 5 groups. One group was the control group with no concentration of ZnS NPs and the other four groups were exposed to 50, 100, 150 and 200 µg/ mL ZnS NPs for 24 hours. At this stage, each concentration of the NPs was individually added to 200 µL of DMEM/F12 medium containing 10% FBS and poured into wells and the cells were treated for 24 hours. The added medium was then discarded and replaced with another DMEM/F12 medium containing MTT (5 mg/ mL in PBS and incubated (37 °C, 5% CO_2 and 90% relative humidity) for 4 hours. The MTT/ PBS solution became yellow and absorbed by the cell due to its net positive charge. Also the potential of the cell membrane that enters into the cells through endocytosis was found on the mitochondria, where it is converted into purple deposits by the mitochondrial succinate dehydrogenase enzyme, which is active only in the living cell respiratory chain. After 4 hours, the medium was aspirated from wells and 100 µl DMSO was added to the plate. Finally, after 15 minutes of shaking the plate on the shaker, the absorbance at 570 nm was measured using an ELISA reader. This MTT assay was repeated three times.

2.9. Data Analysis

All analyses based on the achieved data were performed using Excel software (2010, Microsoft). In order to evaluate the antibacterial effects of ZnS NPs and some antibiotics, including penicillin, amikacin, ceftazidime and primaxin on *Streptococcus pyogenes* and *Acinetobacter baumannii* bacteria in the TSB culture medium, the one-way ANOVA (α= 0.05) was applied. It should be noted that the Kolmogorov–Smirnov test was performed to evaluate the normality of data. The t-test analysis (p <0.05) was used to determine the colony-forming units per milliliter (CFU/mL) and also to calculate the status of *Streptococcus pyogenes* and *Acinetobacter baumannii* bacteria versus the highest concentration of ZnS NPs (150 µg/mL).

3. RESULTS

3.1. SEM Investigation

In order to find the exact size and morphology of synthesized ZnS NPs, an SEM microscope was used. After this experiment, the result showed that the average particle size of synthesized ZnS NPs was about 70 nm. Fig. (**2**) shows related images of ZnS NPs with different magnification. In Fig. (**2a**), magnification is 40000 X and the scale bare is 100 nm and in Fig. (**2b**), magnification is 4000 X and the scale bare is 500 nm. The synthesized ZnS NPs showed dumbbell-shaped and spherical shapes in microscopic studies. Fig. (**2c**) shows the histogram of particle size distribution that was obtained from SEM images; this histogram confirmed that the average particle size of synthesized ZnS NPs was about 70 nm. The size and related morphology of NPs had an impact on the intensity of their anti-bacterial properties.

3.2. Antibacterial Effects of ZnS NPs in MHA Culture Medium

The antibacterial effects of ZnS NPs in MHA culture medium were investigated in the presence of various concentrations of ZnS NPs (50µg/mL, 100 µg/mL and 150 µg/mL). To compare the antibacterial effects of ZnS NPs, penicillin, amikacin, ceftazidime and primaxin antibiotics were used (Table **1**).

The achieved results showed that the antibacterial effects of ZnS NPs had a direct relation to their concentrations. The highest applied concentration of ZnS NPs (150 µg/mL), showed stronger inhibition effects against *Streptococcus pyogenes* and *Acinetobacter baumannii* bacteria.

3.3. Antibacterial Effects of ZnS NPs in TSB Culture Medium

The antibacterial effects of ZnS NPs in TSB culture medium were investigated in the presence of various concentrations of ZnS NPs (50µg/mL, 100 µg/mL and 150 µg/mL). The results of this experiment were achieved through a UV-Vis spectrophotometer (OD: 600 nm). To compare the antibacterial effects of ZnS NPs, penicillin, amikacin, ceftazidime and primaxin antibiotics were used. The obtained results in this experiment are shown in Figs. (**3**-**6**).

According to (Fig. **3**) and (Fig. **4**), it was concluded that the antibacterial effects of ZnS NPs were more effective on *Acinetobacter baumannii*; also, these results confirmed the successful antibac terial effects of ZnS NPs against both bacteria. In the next study, the antibacterial effects of ZnS NPs were compared with some commercial antibiotics (penicillin, amikacin, ceftazidime and primaxin) in the TSB culture medium. In addition, the antibacterial effects of ZnS NPs were compared with distilled water. This experiment was performed for each bacterium separately and the obtained results are provided in Fig. (**5**) and Fig. (**6**).

The statistical analysis of the results was performed based on one-way ANOVA (α= 0.05). The details are provided in Table **2**. As shown in this Table, the maximum effect was found for ZnS NPs (150µg/mL) on *Acinetobacter baumannii*. In addition, other comparable details are also presented.

According to obtained results in Fig. (**5**) and Fig. (**6**), it is clear that there was a high bacterial resistance against the antibiotics used (penicillin, amikacin, ceftazidime and primaxin). In addition, the effects of antibiotics on bacteria were different. In comparison with NPs, bacteria in the presence of antibiotics showed more growth. In the next study, the total bacterial colony/mL was obtained from *Streptococ-*

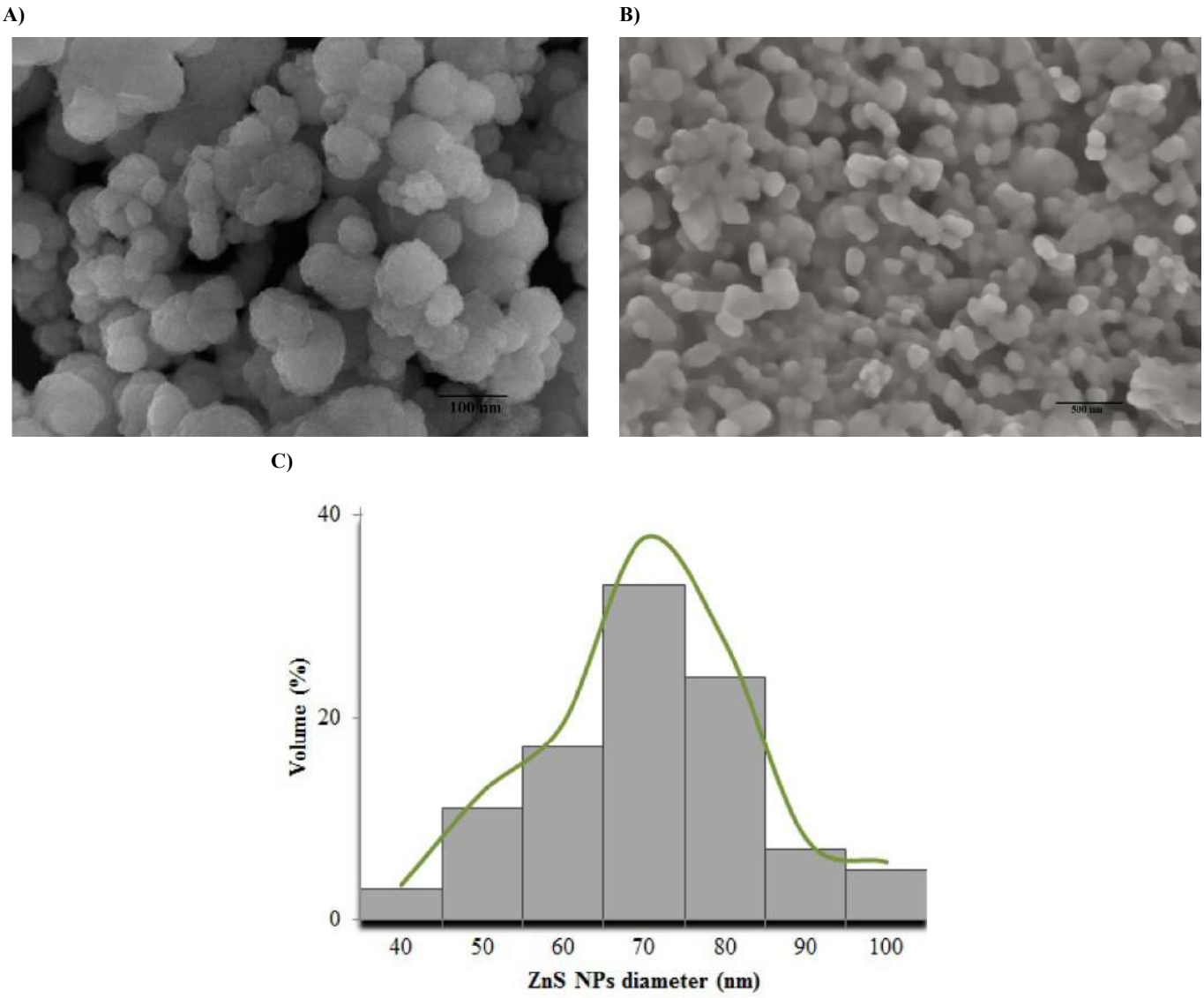

Fig. (2). SEM images of synthesized ZnS NPs with different magnification, **A**): magnification was 40000 X and the scale bare was 100 nm; **B**): magnification was 4000 X and the scale bare was 500 nm; **C**): histogram of ZnS NPs particle size distribution. *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

Table 1. Antibacterial effects of ZnS NPs in MHA culture medium.

Bacterium Type	Effect of ZnS NPs (50µg/mL)	Effect of ZnS NPs (100µg/mL)	Effect of ZnS NPs (150µg/mL)	Effect of Penicillin (200 µg/mL)	Effect of Amikacin (200 µg/mL)	Effect of Ceftazidime (200 µg/mL)	Effect of Primaxin (200 µg/mL)	Effect of Distillated Water
**Streptococcus pyogenes*	+	++	++++	++	+	+	+	-
♠*Acinetobacter baumannii*	++	+++	++++	-	++	+	++	-

+: low antibacterial effect; ++: moderate antibacterial effect; +++ & ++++: high antibacterial effect; -: without any antibacterial effect or near to zero.

* *Streptococcus pyogenes* inhibition Zone Diameter (mm) versus: Effect of ZnS NPs (50µg/mL) = 6; Effect of ZnS NPs (100µg/mL) = 11; Effect of ZnS NPs (150µg/mL) = 17; Effect of penicillin = 10; Effect of amikacin = 5; Effect of ceftazidime = 4; Effect of primaxin = 4; Effect of distillated water = 1.

♠ *Acinetobacter baumannii* inhibition Zone Diameter (mm) versus: Effect of ZnS NPs (50µg/mL) = 8; Effect of ZnS NPs (100µg/mL) = 13; Effect of ZnS NPs (150µg/mL) = 18; Effect of penicillin = 1; Effect of amikacin = 7; Effect of ceftazidime = 5; Effect of primaxin = 6; Effect of distillated water = 1.

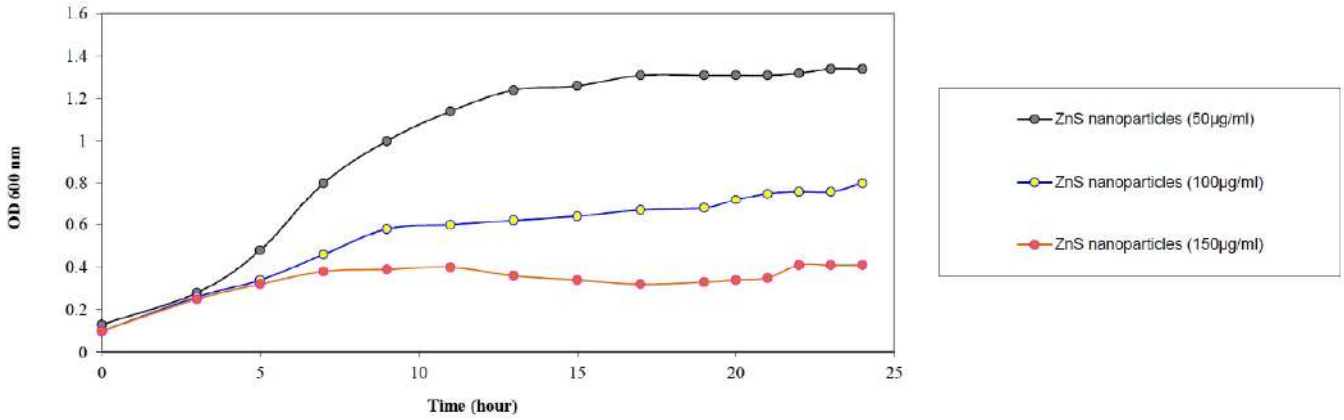

Fig. (3). Effect of ZnS NPs (50µg/mL, 100 µg/mL and 150 µg/mL) on the growth of *Streptococcus pyogenes*. *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

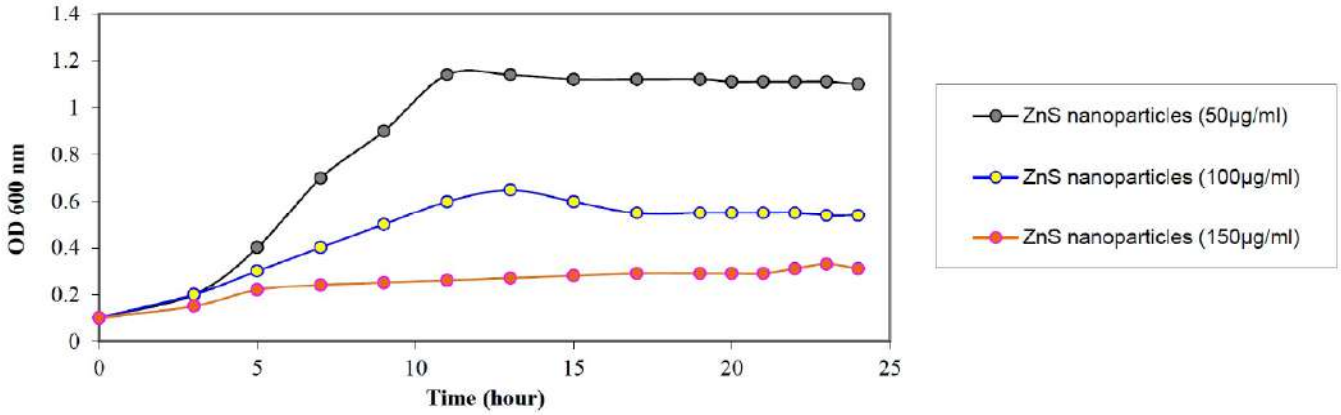

Fig. (4). Effect of ZnS NPs (50µg/mL, 100 µg/mL and 150 µg/mL) on growth of *Acinetobacter baumannii*. *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

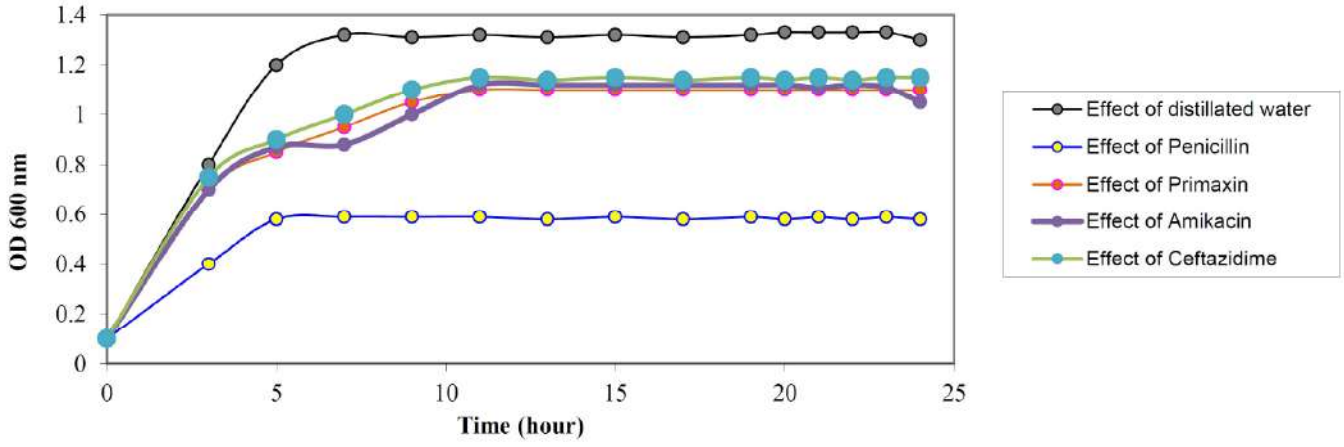

Fig. (5). Effect of penicillin, amikacin, ceftazidime, primaxin antibiotics and distillated water on growth of *Streptococcus pyogenes*. *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

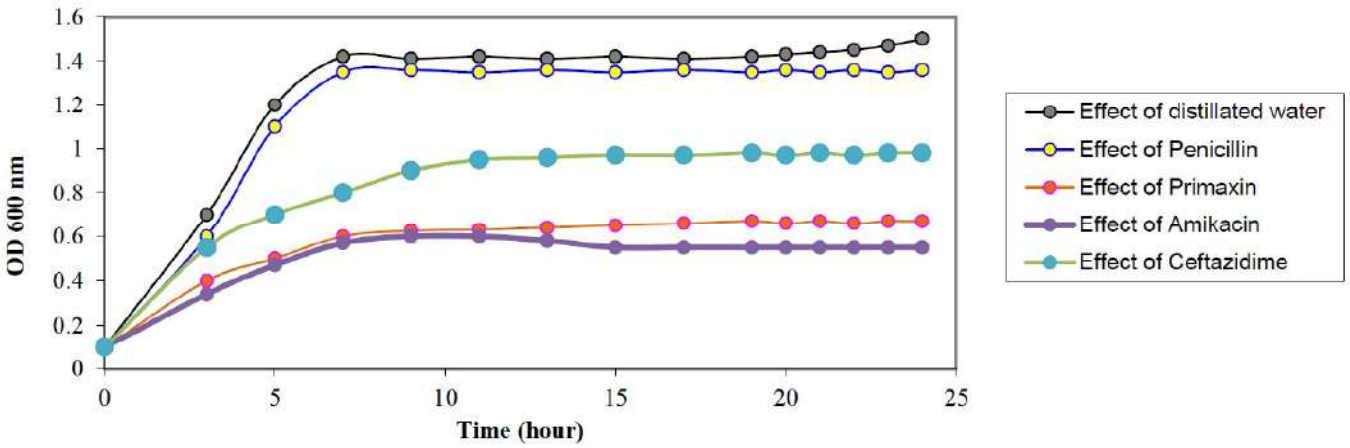

Fig. (6). Effect of penicillin, amikacin, ceftazidime, primaxin antibiotics and distilled water- on the growth of *Acinetobacter baumannii. (A higher resolution / colour version of this figure is available in the electronic copy of the article).*

Table 2. One-way ANOVA analysis of all evaluated groups.

Groups	Total time of Evaluation/ Hour	Sum of Absorbance	Average of Absorbance	Variance of Absorbance
ZnS NPs (50 µg/mL) on *Streptococcus pyogenes*	15	15.57	1.038	0.173346
ZnS NPs (100 µg/mL) on *Streptococcus pyogenes*	15	8.74	0.582667	0.04215
ZnS NPs (150µg/mL) on *Streptococcus pyogenes*	15	5.11	0.340667	0.006392
ZnS NPs (50 µg/mL) on *Acinetobacter baumannii*	15	13.48	0.898667	0.135484
ZnS NPs (100 µg/mL) on *Acinetobacter baumannii*	15	7.18	0.478667	0.025098
ZnS NPs (150µg/mL) on *Acinetobacter baumannii*	15	3.88	0.258667	0.003841
Distilled water on *Streptococcus pyogenes*	15	17.93	1.195333	0.110084
Penicillin (200 µg/mL) on *Streptococcus pyogenes*	15	8.11	0.540667	0.017164
Primaxin (200µg/mL) on *Streptococcus pyogenes*	15	14.65	0.976667	0.07281
Amikacin (200 µg/mL) on *Streptococcus pyogenes*	15	14.66	0.977333	0.075078
Ceftazidime (200µg/mL) on *Streptococcus pyogenes*	15	15.31	1.020667	0.078535
Distilled water on *Acinetobacter baumannii*	15	19.2	1.28	0.1447
Penicillin (200 µg/mL) on *Acinetobacter baumannii*	15	18.06	1.204	0.133454
Primaxin (200µg/mL) on *Acinetobacter baumannii*	15	8.805	0.587	0.023821
Amikacin (200 µg/mL) on *Acinetobacter baumannii*	15	7.66	0.510667	0.016864
Ceftazidime (200µg/mL) on *Acinetobacter baumannii*	15	12.76	0.850667	0.059092

cus pyogenes and *Acinetobacter baumannii* and related results are shown in Fig. (**7**). The results of this experiment were analyzed *via* the t-test (p <0.05) and the mean for *Streptococcus pyogenes* and *Acinetobacter baumannii* was 78500 colony/mL and 88875 colony/mL, respectively.

The cfu/mL was calculated for each bacterium in treatment with various agents. The results showed that the highest used concentration of ZnS NPs (150 µg/mL) was more effective in order to prevent and inhibit the activity of *Streptococcus pyogenes* and *Acinetobacter baumannii* (Most Inhibition Effect). In the last experiment, the viability of *Streptococcus pyogenes* and *Acinetobacter baumannii* bacteria was investigated against the highest concentration of ZnS NPs (150 µg/mL). The viability of *Streptococcus pyogenes* was reached zero at 24 hours after treatment with ZnS NPs (150 µg/mL). While for *Acinetobacter baumannii,* it was 34 hours (Fig. **8**). The mean viability based on t-test analysis for *Streptococcus pyogenes* and *Acinetobacter baumannii* was 1.32 log CFU/ mL and 1.77 log CFU/ mL, respectively (p <0.05).

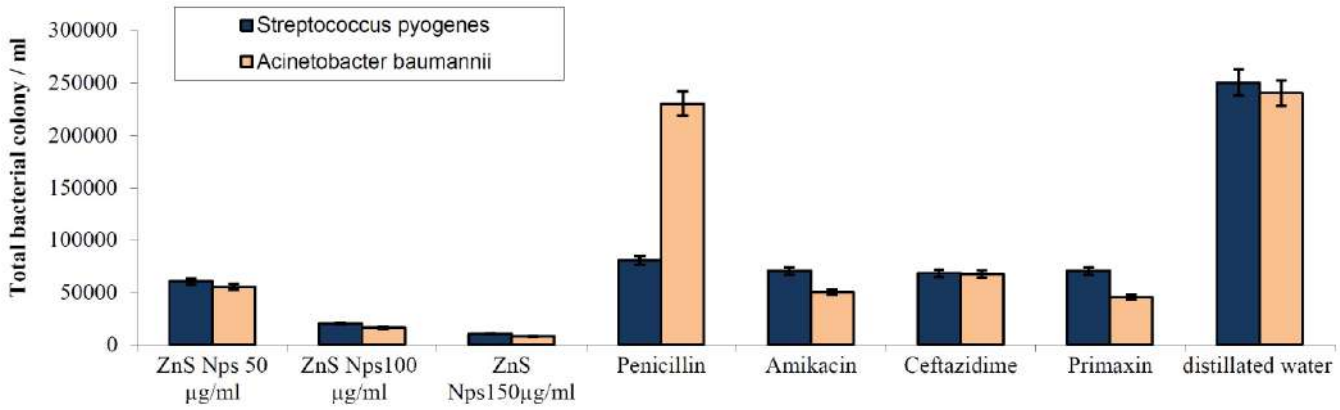

Fig. (7). Total bacterial colony / mL in various conditions: ZnS NPs (50µg/mL, 100 µg/mL and 150 µg/mL), penicillin, amikacin, ceftazidime, primaxin antibiotics and distillated water against *Streptococcus pyogenes and Acinetobacter baumannii* (p <0.05). *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

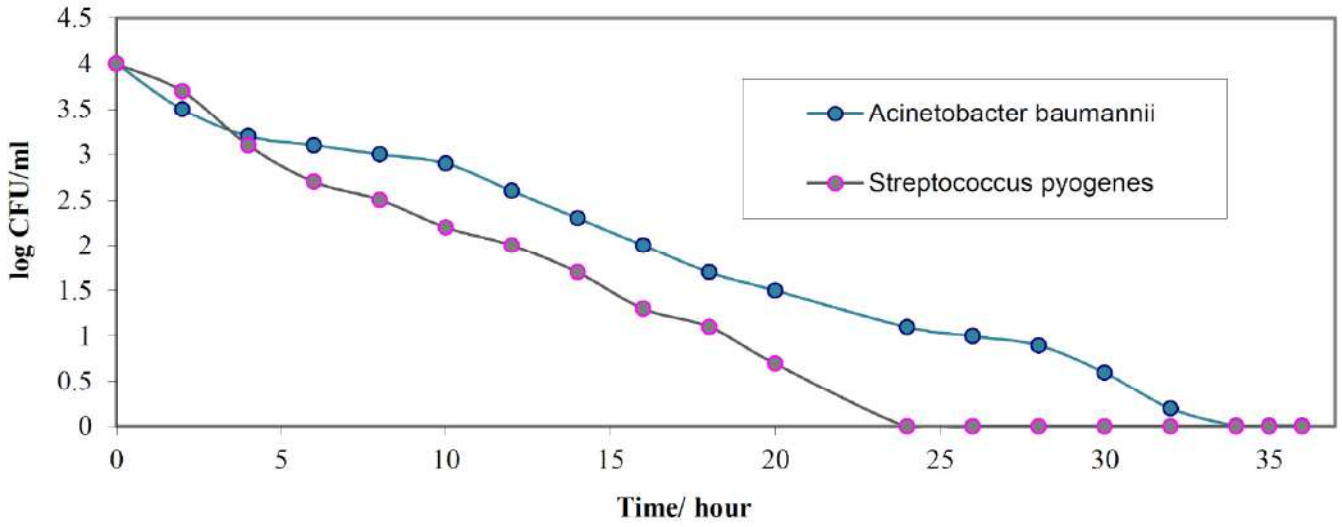

Fig. (8). The maximum concentration effect of ZnS NPs (150 µg/mL) on the viability of Streptococcus pyogenes and Acinetobacter baumannii. *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

3.4. Evaluation of Cell Viability and Cytotoxicity of ZnS NPs using MTT Assay

This experiment was performed based on the mentioned protocols in the methodology section. The results of this assay showed that the cell viability of C6 rat glioma cells that were treated with 50, 100, 150 and 200 µg/mL ZnS NPs was 89%, 91%, 83% and 60% respectively. These results were obtained in the presence of the control group (without any concentration of ZnS NPs), while the cell viability of this was considered 100%. The results confirmed that the safest group (lowest toxicity) was found in the presence of 100 µg/mL ZnS NPs. Along with the increased concentration of ZnS NPs higher than 150 µg/mL, the cell viability was reduced suddenly to 60% that is considered as the major cytotoxicity (200 µg/mL ZnS NPs) among all the investigated groups (Fig. **9**).

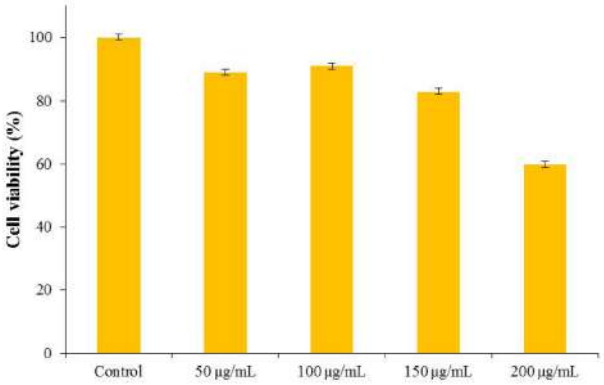

Fig. (9). Cell viability and cytotoxicity of C6 rat glioma cells in the presence of ZnS NPs (50, 100, 150 and 200 µg/mL ZnS). *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

Table 3. Different types of ZnS nanostructures with various synthesis method used as an antibacterial material.

Type of Nano	Average Size of Nanostructures	Synthesis Method	Target Microorganism	References
ZnS quantum dots	2 - 6 nm	Green chemistry-synthesize	*Bacillus cereus, Bacillus subtilis, Klebsiella pneumonia, Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Staphylococcus epidermidis*	[93]
ZnS/chitosan nanocomposite	9 nm	Irradiation technique	*Pseudomonas aeruginosa, Staphylococcus aureus, Candida albicans*	[94]
ZnS NPs	10 - 40 nm	Ultrasonic radiation	*Sordaria fimicola*	[95]
Chitosan-g-poly(acrylamide)/ZnS nanocomposite	19 - 26 nm	Co-precipitation	*Escherichia coli*	[96]
ZnS quantum dots	50 - 80 nm	Chemical vapor deposition	*Staphylococcus aureus, Staphylococcus epidermidis, Peptostreptococcus anaerobius, Streptococcus pyogenes, Bacteroidesfragilis, Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa*	[97]
Gadolinium (Gd) doped ZnS NPs	3.1 - 3.8 nm	Microwave irradiation method	*Candida albicans, Aspergillus flavus, Bacillus subtilis, Klebsiella pneumonia, Escherichia coli, Pseudomonas syringae*	[98]
Core-shell ZnS/SiO_2	5 - 8 nm	Chemical precipitation	*Bacillus subtilis, Staphylococcus aureus, Salmonella typhi Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa*	[99]
ZnS quantum dots	2 - 3 nm	Co-precipitation	*Klebsiella pneumoniae, Escherichia coli, Staphylococcus aureus, Bacillus thuringiensis*	[100]
ZnSe@ZnS quantum dots	4.8 nm	Chemical	*Escherichia coli, Staphylococcus aureus*	[101]
CdSe@ZnS quantum dots	5.74 nm	Chemical	*Acinetobacter junii, Bacillus cereus, Flavobacterium columnare, Pseudomonas aeruginosa*	[102]
Cellulose nanocrystals (CNCs)/ ZnS quantum dots	15 nm	Hydrothermal method	*Escherichia coli*	[103]
ZnS–ZnO nanocomposite	2 μm	Hydrothermal method	*Escherichia coli*	[104]
CdSe/ZnS NPs	10 nm	Chemical	*Escherichia coli, Bacillus subtilis*	[105]
ZnS nano Composite	15 - 30 nm	Chemical deposition	*Peptostreptococcus Anaerobius, Streptococcus Pyogenes, Bacteroides fragilis, Escherichia coli, Klebsiella pneumonia*	[106]
ZnS NPs	100 nm	Sonochemical precipitation	*Candida albicans*	[107]
Copper doped ZnS (Cu:ZnS) NPs	~63 nm and ~85 nm	Solvothermal	*Escherichia coli, Bacillus subtilis*	[108]
PANI Coated ZnS Nanocomposite	15.7 nm and 15.4 nm	Micro-wave-assisted solvothemal	*Escherichia coli, Proteus mirabilis*	[109]

(Table 3) contd….

Type of Nano	Average Size of Nanostructures	Synthesis Method	Target Microorganism	References
ZnS nanocrystals	3 - 5 nm	Co-precipitation	*Escherichia coli, Klebsiella pneumonia, Bacillus subtilis, Staphylococcus aureus*	[110]
ZnS nanospheres	100 - 400 nm	Chemical	*Escherichia coli*	[32]
ZnS-cellulose nano-composite	10 nm	Co-precipitation	*Escherichia coli*	[111]
ZnS NPs	70 nm	Co-precipitation	*Streptococcus pyogenes, Acinetobacter baumannii*	This work

4. DISCUSSION

Despite the fact that we live in an era full of new and advanced technologies, and with these technologies, we can realize the mechanisms of the disease and can design new drugs in the molecular forms, infectious disease is considered one of the worldwide health challenges [74, 75]. At the beginning of the twentieth century, infectious diseases were the leading cause of mortality [76, 77]. Reduced mortality and infectious diseases in the past century were primarily due to the use of antimicrobial agents [78]. Yet today, resistance against antibiotics has been reached to a high level and this resistance makes antimicrobial drugs inapplicable in some cases [26, 79]. Unfortunately, there is no certainty that the growth and development of new antimicrobial drugs can be realized in a timely manner for rapid and permanent improvement in antibacterial effects against microbial pathogens [80]. For example, drug-resistant infections in hospitals and in communities that are caused by pathogenic gram-positive and gram-negative bacteria are prevailing and the steady development of antimicrobial resistance will threaten human health due to the inability of humans to fight against these infections [81]. The dynamic patterns of infectious diseases and the occurrence of resistant bacteria types against a number of antibiotics are raised as challenging issues [80, 82, 83]. One of the recent efforts to eliminate this challenge is discovering the antimicrobial effects of nanomaterials, in the presence ofwhich , the microbial pathogens may not be able to raise bacterial resistance [84-89]. Preparation of antimicrobial NPs compared to the synthesis of antibiotics is affordable and also stable for long-term storage with long half-life sufficiently [90, 91]. In addition, many NPs can be stable in poor conditions [92]. So far, the ZnS nanostructures have been used to fight against several bacteria with appropriate size, morphology and production methods being applicable and useful to enhance their antibacterial effects (Table **3**).

The high surface area to volume ratio and physical and chemical characteristics of various nanomaterials also contribute to their effective antimicrobial activity [112, 113]. Destruction of the cell wall and subsequent degradation of the cell membranecause disruptions in the activities of proteins and DNA that are probable mechanisms for antibacterial effects of ZnS NPs against *Streptococcus pyogenes* and *Acinetobacter baumannii* NPs.

CONCLUSION

Here, ZnS NPs with the average size of 70 nm were synthesized and applied as an antibacterial agent against *Streptococcus pyogenes* and *Acinetobacter baumannii* in the presence of several major antibiotics including penicillin, amikacin, ceftazidime and primaxin. Based on UV-Vis investigations (OD: 600 nm), the antibacterial effect of ZnS NPs showed a direct relation with the applied concentrations and the maximum effect was obtained when the concentration of ZnS NPs was 150 µg/mL. Total bacterial CFU of *Streptococcus pyogenes* and *Acinetobacter baumannii* bacteria in the maximum concentration of ZnS NPs (150 µg/mL) was 10000 colony/mL and 8000 colony/mL respectively. It should be noted that the maximum antibacterial effects of antibiotics (200 µg/mL) were observed to be related to primaxin where the total bacterial CFU of *Streptococcus pyogenes* and *Acinetobacter baumannii* bacteria were 70000 colony/mL and 45000 colony/mL respectively and was not comparable against ZnS NPs. The viability of *Streptococcus pyogenes* and *Acinetobacter baumannii* bacteria in the presence of ZnS NPs (150 µg/mL) was 24 hours and 34 hours, respectively. The findings of this research can be used in the future of medical treatment platforms in order to reduce the infectious diseases caused by *Streptococcus pyogenes* and *Acinetobacter baumannii*.

AUTHOR CONTRIBUTIONS

Masoud Negahdary designed the study. Zeinab Morshedtalab and Ghasem Rahimi conducted the experiments. Zeinab Morshedtalab, Asieh Emami-Nejad, Alireza Farasat, Azita Mohammadbeygi and Nahid Ghaedamini analyzed the results. All authors contributed to writing the final manuscript. The final manuscript was approved by all authors.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are base of this research.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

FUNDING

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

We would like to thank all of our colleagues who provided insights and expertise that greatly assisted in the research work.

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