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Evaluate Activity of Chitosan Nanoparticles (CHNPS) as Antibacterial Against Some MDR Pathogenic Bacteria

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Abstract:

Chitosan nanoparticles (ChNPs) are recognized to show antibacterial action against a wide spectrum of diseases in this regard. As a result, they have emerged as a promising contender for combating multi-drug resistance.

Gram-positive and Gram-negative bacteria were obtained from infected burns and wounds. The antibacterial activity of ChNPs was performed using the disk diffusion method in various doses (500, 250, 125, 62.5, and 31.25 g/ml). Each isolate's MIC and MBC were also measured. ChNPs have shown dose-dependent antibacterial activity, and have even surpassed certain antibiotics in terms of efficiency. The MICs of ChNPs ranged from 125 to 62.5 g/ml, whereas the MBCs ranged from 250 to 500 g/ml. When GNPs were employed at the correct concentration, bacterial growth was significantly halted. ChNPs are recommended as a low-cost antibacterial option, particularly for treating ectopic infections without the danger of bacteria.

Keywords: Antibacterial activity, Chitosan Nanoparticles, MIC.

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1. INTRODUCTION

Recent years have seen a lot of interest in the emergence of antibiotic-resistant bacteria. According to reports, no antimicrobial agent has ever been used on a microbe without encountering resistance [1]. This recent trend has compelled researchers to create novel antimicrobial substances that have an impact on the pathogenic bacteria that are becoming more and more resistant to antibiotics [2]. In this context, chitosan has recently gained popularity because it has not been observed that bacteria may get resistant to it [3].

The exoskeleton of crustaceans as well as many other creatures, such as insects and fungi, includes chitosan, or (1-4) 2-amino-2-deoxy-D glucan, which is produced through chitin deacetylation [4]. Over the past 20 years, researchers have become interested in chitosan due to its advantageous features. As an illustration, it is non-toxic, biocompatible, palatable, and also has antibacterial qualities [5-8]. Chitosan has been explored as a medication delivery technology and in biological applications for many years [9–11]. The majority of current research has focused on chitosan's antimicrobial action in liquids, gels, films and fibers [12–16]. Chitosan nanoparticles (CSNPs) are being used with increasing attention, primarily because of their reliable polymeric and cationic characteristics [17]. Numerous research teams have studied various methods for producing chitosan nanoparticles, including emulsion crosslinking [18], ionic gelation [19], emulsion-droplet coalescence [20], the reverse micellar method [21], desolvation [22], nanoprecipitation [23], spray drying [24], the emulsion solvent diffusion method [25], and modified ionic gelation with radical polymerization [26]. Because of their tiny size, high surface-to-weight ratio, accessibility, low toxicity, and antibacterial qualities, CSNPs has been employment in many biomedical areas. While several ideas have attempted to explain chitosan's antibacterial properties, the precise processes remain a mystery. These ideas include intracellular leakage, in which negatively charged bacterial surfaces like lipopolysaccharides (LPS) attach to positively charged chitosan [27, 28]. Due to this

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binding, the permeability of the bacterial membrane has changed, resulting in the release of intracellular components and cell death [29]. When compared to chitosan, chitosan nanoparticles (CNPs) showed greater antimicrobial activity [30–33]. This shows that ChNPs may have the ability to fight infections as an antibacterial. As a result, the goal of this work is to assess the antibacterial activity of chitosan nanoparticles (ChNPs) as well as the MIC and MBC.

2. MATERIAL AND METHODS

Bacteria Isolation

The tested bacteria were isolated from burns and wounds at Hillah Teaching Hospital, Iraq. All samples were isolated and purified using conventional bacteriological methods. The Vitek-2 compact system (Biomérieux) was used to confirm all of the isolates.

Antibiotic susceptibility

Five different antibiotics were tested on Mueller-Hinton agar plates (Carl Roth, Germany) using the disk-diffusion technique following (CLSI) and inhibition zones were measured [34].

Antibacterial Activity of ChNPs

The antibacterial activity of ChNPs was tested on bacteria grown on nutrient agar slants [34] using five dilutions of ChNPs (500, 250, 125, 62.5, and 31.25 g/ml) then the isolates were incubated overnight at 37°C then the inhibition zone diameter was measured.

MIC and MBC determination

The same antibacterial activity was followed in addition to the negative control was used. Each isolate was tested in triplicates. The MIC was determined by spectrophotometry (600nm) for tubes with no turbidity while MBC was determined by the concentration that showed no growth [35].

3. RESULTS AND DISCUSSION

Isolated Pathogenic Bacteria

Four bacterial isolates were identified using Vitek-2 compact system (Biomérieux) two Gram-negative and two Gram-positive.

Susceptibility of the isolated bacteria

According to the CLSI, all bacterial isolates found resistant to all tested antibiotics (Figure 1).

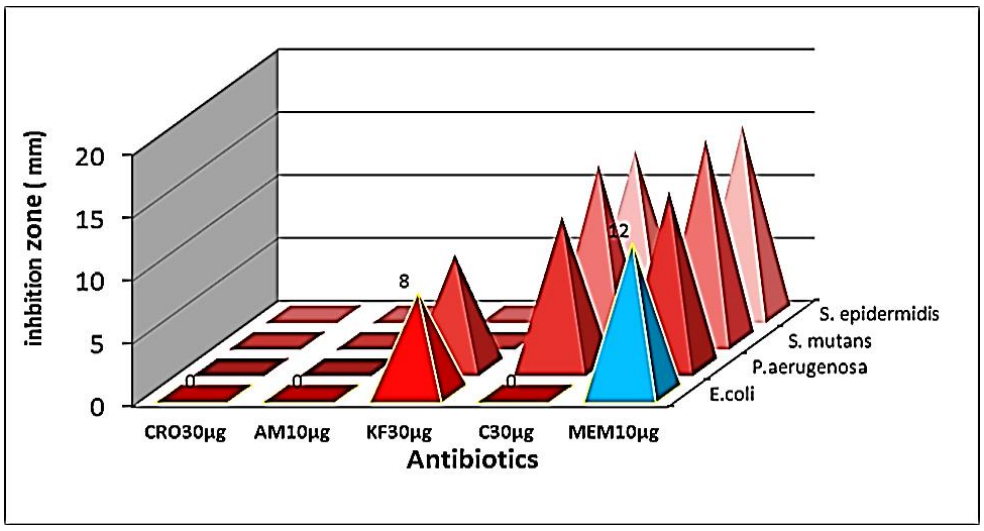

Figure 1: *E. coli*, *P. aeruginosa*, *S. epidermidis*, and *S. mutans* antibiotic sensitivity. CRO: Ceftriaxone, AM: Ampicillin, KF: Cephalothin, C: Chloramphenicol, MEM: Meropenem

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According to the findings of the study, all bacterial isolates were resistant (100%) to ceftriaxone and ampicillin, 75% to chloramphenicol, 50% to cephalothin,, and 0% to meropenem [36].

ChNPs antibacterial activity

The inhibition zone is achieved in different dimensions while estimating the antibacterial activity of ChNPs against several harmful bacteria (*E. coli*, *P. aeruginosa*, *S. epidermidis*, and *S. mutans*) (Figures 2-5)

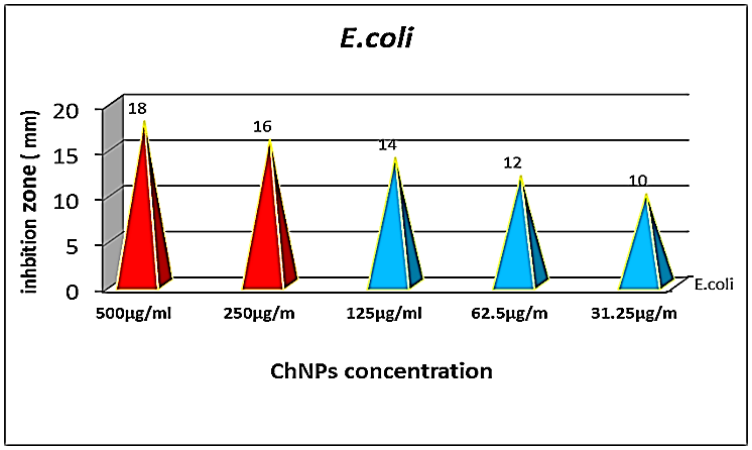

Figure 2: Antibacterial activity of ChNPs on *E. coli*

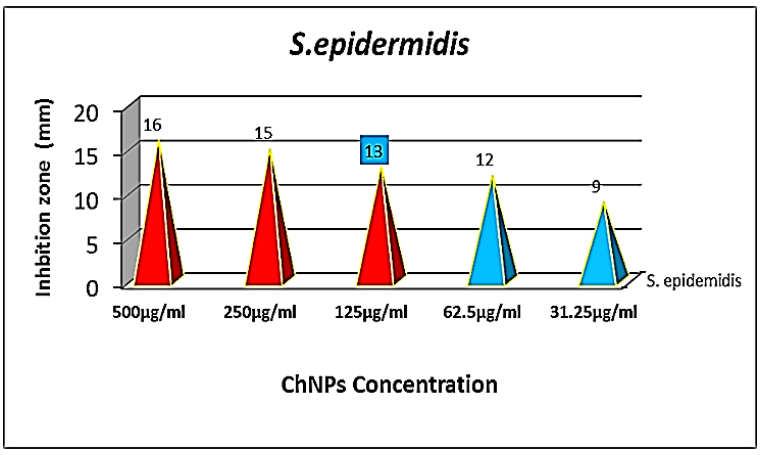

Figure3: Antibacterial activity of ChNPs on *S. epidemidis*

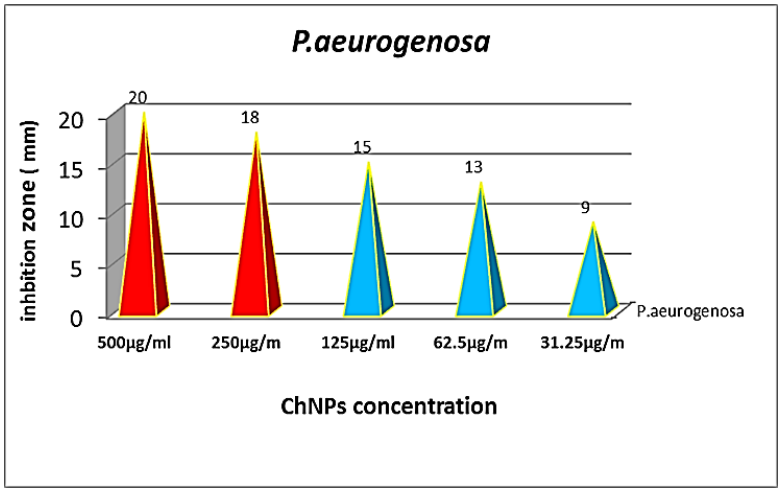

Figure 4: Antibacterial activity of ChNPs on *P. aeurogenosa*

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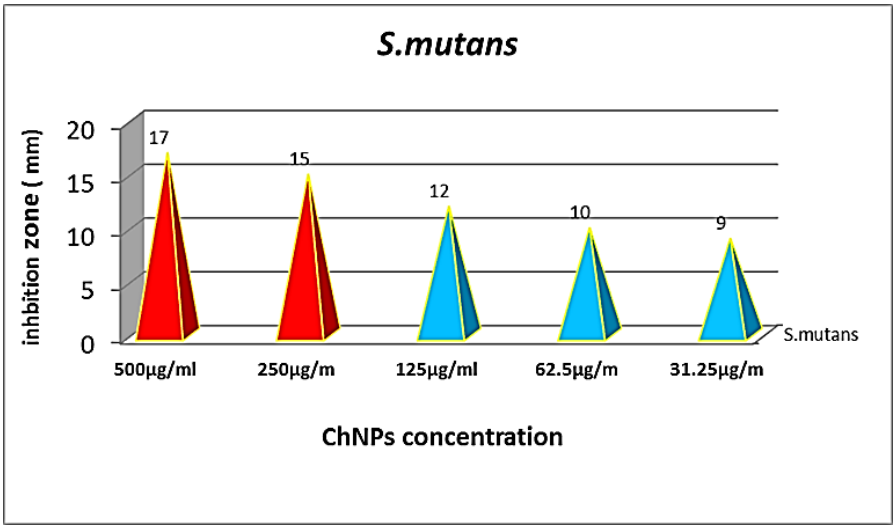

Figure 5: Antibacterial activity of ChNPs on *S. mutans*

ChNPs showed increased inhibitory zone width with increasing ChNPs concentration, even exceeding the activity of chosen antibiotics. The largest zone of inhibition was observed against all bacterial isolates at 500 g/ml concentration, 20mm zone of inhibition against *P. aeurogenosa* and *S. epidermidis* being the least sensitive isolate to the ChNPs. Figures 6 and 7 show that ChNPs suppress the growth of all microorganisms under study in a dose-dependent way. Many processes contribute to ChNPs' antibacterial action. The first process is based on ROS production, followed by oxidative stress, which causes protein chemical damage and genetic material (DNA) in bacteria. Second, physical damage due to electrostatic interactions with the cell membrane proteins leading to cell death. Another study found that the nanoparticles' tiny size can aid ChNPs' antibacterial effectiveness [37].

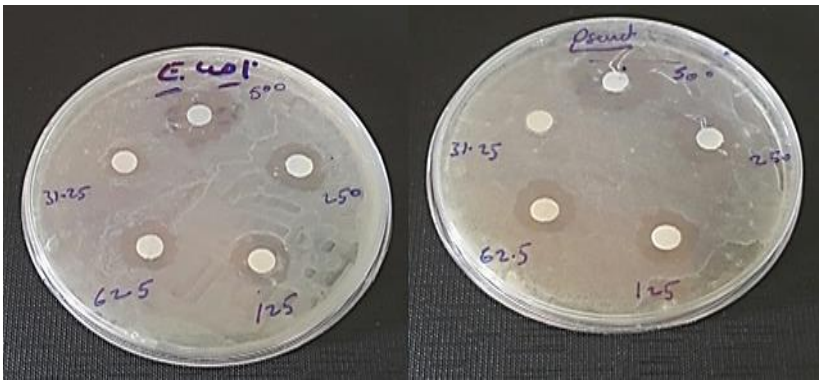

Figure 6: Gram-negative bacterial growth inhibition as a result of ChNPs

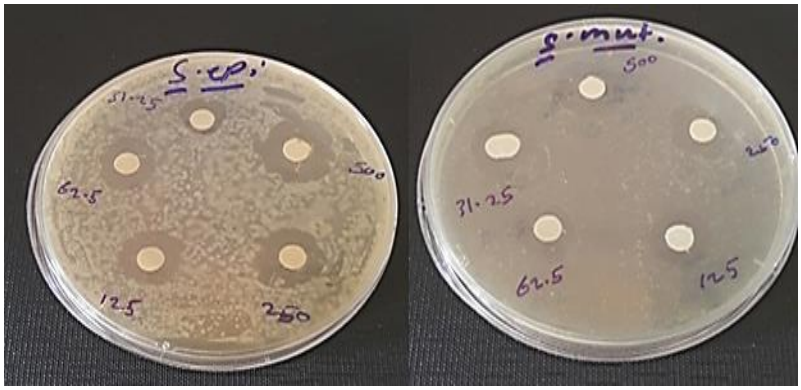

Figure 7: Gram-positive bacterial growth inhibition as a result of ChNPs

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Because of their activity against pathogenic bacteria, it is conceivable to employ ChNP to treat diverse infections (in vivo) as an alternative treatment. The lowest inhibitory concentration (MIC) was used to assess the antibacterial activity of ChNPs. In Table (1), The MIC value supports the inhibition zone test results, where ChNPs had increased activity against both Gram-negative and Gram-positive bacterial isolates as compared to previous research [38].

Table 1: ChNPs MIC and MBC (µg/ml)

Bacterial Isolates	ChNPs MIC	ChNPs MBC
E. coli	**125**	**500**
P. aeruogenosa	**62.5**	**500**
S. mutans	**62.5**	**250**
S. epidermidis	**125**	**500**

The MIC and MBC of ChNPs, varied from 62.5 to 125 g/ml, and the MBC ranged from 250 to 500 g/ml [39, 40].

4. CONCLUSION

Chitosan nanoparticles are distinguished by their possible antibacterial action. The antimicrobial efficacy of chitosan nanoparticles against therapeutically important bacteria seemed promising. ChNPs were found to be effective against *S. mutans, P. aeruginosa, S. epidermidis,* and *E. coli.* To establish chitosan nanoparticles as an antibacterial agent, more research on their toxicity is required.

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