Wasit Journal for Pure Science

Journal Homepage: https://wjps.uowasit.edu.iq/index.php/wjps/index
e-ISSN: 2790-5241 p-ISSN: 2790-5233

Antimicrobial Activities of Chitosan and Nanochitosan Against Aerobic Infection in Wound

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DOI: https://doi.org/10.31185/wjps.680
Received 06 January 2025; Accepted 12 February 2025; Available online 30 March 2025

ABSTRACT: In this present study, aerobic bacteria were isolated from wound and burn samples taken from diabetic foot patients at Al-Salam Teaching Hospital. Chitosan, which comes from insects and arthropods, was employed as an antibacterial agent. It binds to the negatively charged bacterial cell wall, producing damage and alterations in permeability. The concentration of 1% was discovered to be the most efficient against aerobic bacterial infections. The degree of deacetylation, which directly effects the positive charge density, has a significant effect on the antibacterial activity of chitosan and its derivatives.

Keywords: *pseudomonas aeroginosa*; chitosan; Nano chitosan, Antibacterial activity; The degree of deacetylation

1.INRODUCTION

Wound infections are a leading cause of surgical patient death and one-third of nosocomial infections, especially in developing nations [1]. These infections are caused by aerobic bacteria such as Pseudomonas aeroginosa. Natural polymers such as chitosan provide a fresh strategy to combating bacterial infections since they are biocompatible, non-toxic, and inexpensive [2]. Chitosan is biodegradable, non-toxic, and promotes wound healing, making it an ideal foundation for wound dressings. The features of extracted chitosan are largely impacted by its initial source and the deacetylation procedure [3] One of the most common polymers employed in the field of nano medicine delivery systems is nano chitosan [4]) Nanoparticles, such as nano chitosan, have been a prominent focus in medication delivery, with research indicating that smaller particles are more effective than bigger ones [5]. The study aims to isolate and identify aerobic bacteria from individuals with wound and burn diseases and testing the antibacterial activity of chitosan and nano chitosan at different dilutions on bacterial isolates.

2.MATERIAL AND METHOD

2.1. Sample collection

Between the end of September 2023 and May 2024, 100 pus swabs were collected from patients with wound infections and burns of both sexes, ranging in age from 6 to 76 years old. They were recognized using Gram staining, as well as culture and biochemical characteristics, and *pseudomonas aeroginosa* was isolated and identified on cetrmide agar. Biochemical assays such as oxidase and catalase were used to validate the isolated colonies [6]. The isolates were kept at -70 degrees.

2.2 Determination of degree of deacetylation (DDA)

Chitosan is a commercially available polysaccharide created via deacetylation of chitin, which results in amino groups. Chitin and chitosan are distinguished by the degree of deacetylation (DD), which is measured using FTIR spectroscopy [7]

2.3The preparation of concentration

The preparation of concentration to make chitosan, dissolve 1g in 1% acetic acid, then dilute with distilled water. Chitosan solutions were produced at several concentrations (0.5%, 1%, and 2%) for use as antibacterial agents. Acetic acid was employed as a control; concentrations less than 0.5% did not inhibit any of the bacteria tested.

2.4 Preparation of chitosan nanoparticle:

Nanochitosan was obtained from arminano. The nano chitosan solution was prepared by dissolving 10 mL of chitosan in 90 mL of distilled water. The dissolving process took place at room temperature. To produce a 2% concentration of micro chitosan, 80 mL of distilled water was added to the solution, and then stirred for another 6 hours [8]

2.5 Antibiotic sensitivity test

The Kirby-Bauer method was employed to administer the test. Pure colonies were introduced to test tubes with 5 ml of Nutrient broth medium and cultured at 37 ºC for 2-8 hours or until turbidity occurred. The results were compared to a McFarland standard tube of 0.5 concentration. To alter the tube density, a sterile physiological saline solution was utilized to match the density of the McFarland tube. Bacteria were dispersed over Muller Hinton agar with sterilized cotton swabs in an L-shape. Antibiotic discs were dispersed using sterile forceps and incubated at 37ºC for 24 hours. The findings were analyzed by measuring the diameter of the inhibitory zone and comparing it to the reference discs.

2.6 Minimum inhibitory concentration (MIC):

The agar well diffusion experiment [9] was used to assess chitosan samples' antibacterial effectiveness against pathogenic microorganisms. Chitosan and nanochitosan samples were dissolved in 1% acetic acid (0.01g/mL), followed by three concentrations of chitosan and nanochitosan with twofold dilution (1%, 2%, and 0.5%). All plates containing aerobic bacteria had wells of similar size and depth, with a hole diameter of 6 mm, punched in the agar with a cork borer. Each well was filled with 100µl of various research agents. After 20 minutes at room temperature, the plates were incubated at 37°C for 24 hours. The presence of an inhibition zone indicates susceptibility to the tested chemical, whereas the absence of an inhibition zone indicates resistance. For antimicrobial sensitivity tests, the bacterial suspension of the organism should be equivalent to the 0.5 McFarland standard.

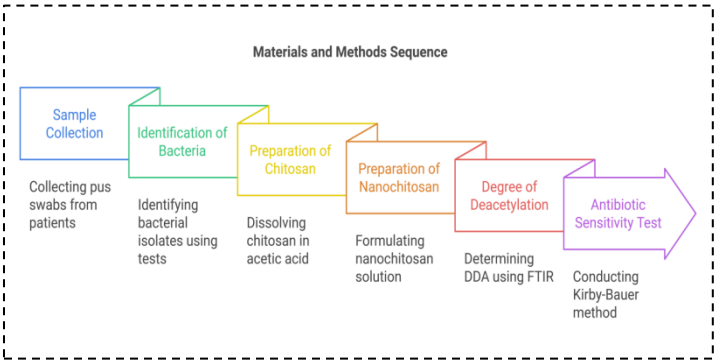

Figure (1): Material and Method sequence

3. RESULT AND DISCUSSION

3.1. Isolation and characterization of aerobic bacteria from wound patients

The purpose of this study was to identify and characterize pseudomonas aeroginosa from individuals with wounds. These bacteria have been linked to the onset and progression of infection, and knowing their biochemical properties is critical for a full understanding of their involvement in the disease.

3.2 Isolation and Characterization of the *pseudomonas aeroginosa*

Gram-negative bacteria are commonly seen in infectious illnesses. In nosocomial illnesses like Pseudomonas aeruginosa, isolates outnumber other bacterial species. Bacterial isolates were purified using optimal medium and tested for oxidase-positive and lactose fermentation on MacConkey's agar, as well as hemolysis on blood and cetrimide agar. [10] as shown in figure (2) It produced colors like blue and green pigments during Muller fermentation.

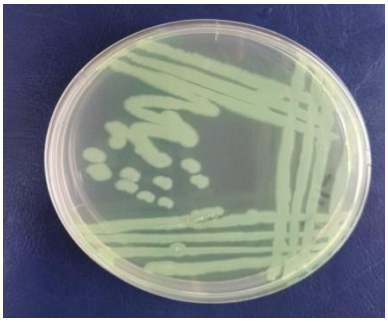

Figure (2). *pseudomonas aeroginosa on cetrimide agar*

3.3 Samples isolates distribution

3.3.1 According.to the bacterial growth.

The increasing frequency of bacterial infections and their resistance to antibiotics needs research into the bacterial species that cause resistance. This will help solve the problem. A total of 100 samples were collected from persons of all ages and genders with chronic infections such as surgical wounds, burns, boils, diabetic foot ulcers, and clostridial soft-tissue infections. Both individuals were outpatients and were admitted to Mosul's Al-Salam General Hospital. Out of ten samples collected from persons suffering from the illness, 91 (91%) showed positive bacterial growth in various cultured conditions, whereas 9 (9%) showed no growth (negative cultures) as shown in table (1)

The patient has taken antibiotics, for example, and the growth is not always obvious. These bacteria "must be cultured in their natural environment," because they cannot thrive in a laboratory setting. They do, however, occasionally transmit illnesses. [11]

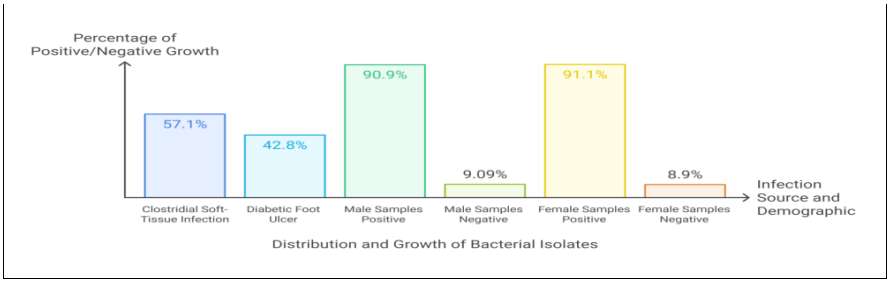

Figure (3). Distribution and Growth of bacterial isolates

Table (1): Distribution According.to the bacterial growth

Type of growth	Number	Percentage
Positive culture	**91**	**91%** **91%**
Negative culture	**9**	**9%**

		9%
Total	**100**	**100%**

3.3.2. According to the sources of the infection

According to the source of the infection (clostridial soft tissue and burns infection, diabetic foot and wounds), the bacterial isolates that showed positive growth were 18 (19.8%), 22 (24.2%), 25 (27.4%), and 26 (28.6%), while negative growth of bacterial isolates appeared in 2 (22.2%), 2(22.2%), 4(44.4%), and 1(11.2%) sample, respectively (Table 2).

Table 2. The samples distribution according to sources of collection.

Sources types	Growth (+)	%	Growth (-)	%	Total	%
Clostridial Soft Tissue infection	18	19.8	2	22.2	20	20%
Burns Infection	22	24.2	2	22.2	24	24%
Diabetic foot	25	27.4	4	44.4	29	29%
Wounds	26	28.6	1	11.2	27	27%
Total	91	100	9	100	100	100

According to [12] positive bacterial growth in 96.9% of samples showed a surgical wound demonstrated 86.9% positive development of bacterial infections in post-caesarean wounds.

3.3.3 According to Sex.

Bacterial infections may harm everyone, regardless of age or gender. Bacterial growth was seen in samples from various sources and sex groups. (Table.3). Female samples consisted of 41 samples, which were less prominent than male samples (50). Male samples showed positive growth cultures of bacterial isolates. 50 (90.9%) samples showed no growth, whereas 5 (9.09%) samples did. The female with positive growth from the bacterial isolates was at 41 (91.1%), while the non-growth samples were at 4 (8.9%).

Table 3. The samples number and percentage according to Sex.

The Gender	Growth(+)	%	Growth(-)	%	Total
Male	50	90.9	5	9.09	55
Female	41	91.1	4	8.9	45
Total	91	-	9	-	100

According to the survey, male patients are admitted to hospitals more frequently than female patients, resulting in a lopsided patient population. The findings were similar to those of most ailments have unequal distributions among male and female persons. Females tend to be less vulnerable to infection than males in infectious diseases, however this varies. This dimorphism might be explained by factors such as lifestyle, professional exposure, recreational activities, and access to healthcare. Sex dimorphism can also be regulated by X-linked variability and sex hormone levels. Gender differences are generated by environmental factors, whereas "sex" refers to biological dimorphism [13]

3.3.3 Distribution of bacterial isolates according to the infection sources:

The results of aerobic pseudomonas aeroginosa bacteria, revealed that the case clostridial soft-tissue infection was 6 (16.2%), diabetic foot ulcer was 10 (27.02%), burn was 9 (24.3%), and wound was 12 (32.4%). Many variables, such as

lifestyle, socioeconomic level, geographical dispersion, age, and gender, are likely to impact the frequency and variety of bacteria found in clinical samples. the results agreed with [14]

Table (4) The number of bacterial isolates according to the infection sources

Bacteria	Burns Infection	Clostridial Soft-Tissue	Diabetic foot ulcer	Wound	Total
Pseudomonas aeroginosa	9	6	10	12	37
Other bacteria	21	13	15	32	81
Total	30	19	25	44	118

3.3.4 Antibiotic Susceptibility test:

Nine antibiotic discs were used to test the sensitivity of numerous bacterial isolates from diverse human body illnesses, as. Using the Kirby-Bauer Method test, the *pseudomonas aeroginosa* show resist to the antibiotics as shown in figure (4)

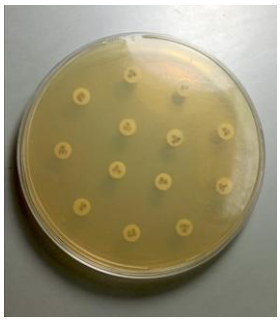

Figure (4) Antibacterial activity against *pseudomonas aeroginosa*

3.3.5 Minimum Inhibitory Concentration (MIC) of Chitosan against bacterial isolate

Chitosan, a cationic natural polymer, has antibacterial properties against pathogenic microorganisms. Inactivation experiments in a laboratory medium revealed sensitivity to chitosan at concentrations of 0.1%, 2%, and 0.5%, with a minor inhibitory effect (28.75%) as shown in figure (5) Its antibacterial properties come from its ability to efficiently transfer extrinsic antimicrobial chemicals into the affected area. The exact mechanisms of chitosan's antimicrobial actions are unknown, but it has been proposed that the interaction of positively charged chitosan molecules with negatively charged microbial cell membranes disrupts microbial membranes, allowing proteinaceous and intracellular components to leak out. Chitosan has outstanding antibacterial properties against both Gram-negative and Gram-positive bacteria. The method of action varies between G+ and G- bacteria due to variations

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3.3.6 Minimum Inhibitory Concentration (MIC) of nanochitosan against bacterial isolate

Nanochitosan inhibited all pseudomonas spp isolates except at 0.5% (42%). As a consequence, the study provides strong evidence of chitosan nanoparticles' efficacy as antibacterial agents. The smaller size of the chNPs alters the mechanism of antibacterial activity, allowing them to easily pass through membrane barriers and disrupt the microorganism's physiological and chemical activities as shown in figure (6).

Table (5): Minimum Inhibitory Concentration (MIC) of chitosan against *pseudomonas perfringens.*

Number of Sample	Chitosan 0.5%	Chitosan 1%	Chitosan 2%
1	S	S	S
2	R	S	S
3	R	S	S
4	S	S	S
5	R	S	S
6	R	S	S
7	R	S	S

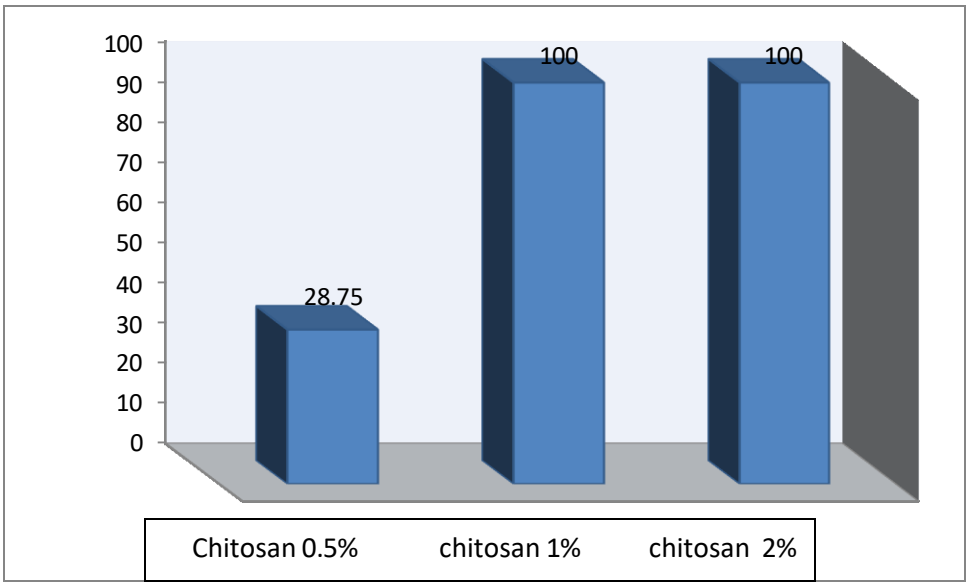

Figure (5). Minimum Inhibitory Concentration (MIC) of chitosan against *pseudomonas perfringens*

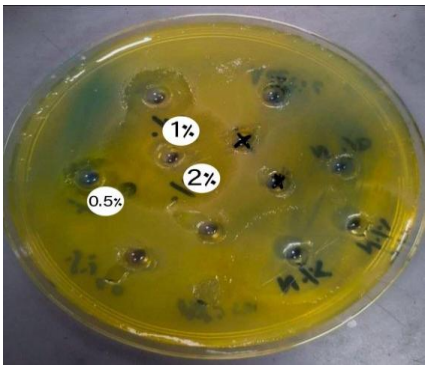

Figure (6). Minimum inhibitory concentration (MIC) of chitosan for *pseudomonas* sp.

Table (6): Minimum Inhibitory Concentration (MIC) of nano chitosan against *Pseudomonas aeruginosa*

Number of Sample	ChNP 0.5%	ChNP 1%	ChNP 2%
1	R	S	S
2	R	S	S

3	R	S	S
4	S	S	S
5	S	S	S
6	R	S	S
7	S	S	S

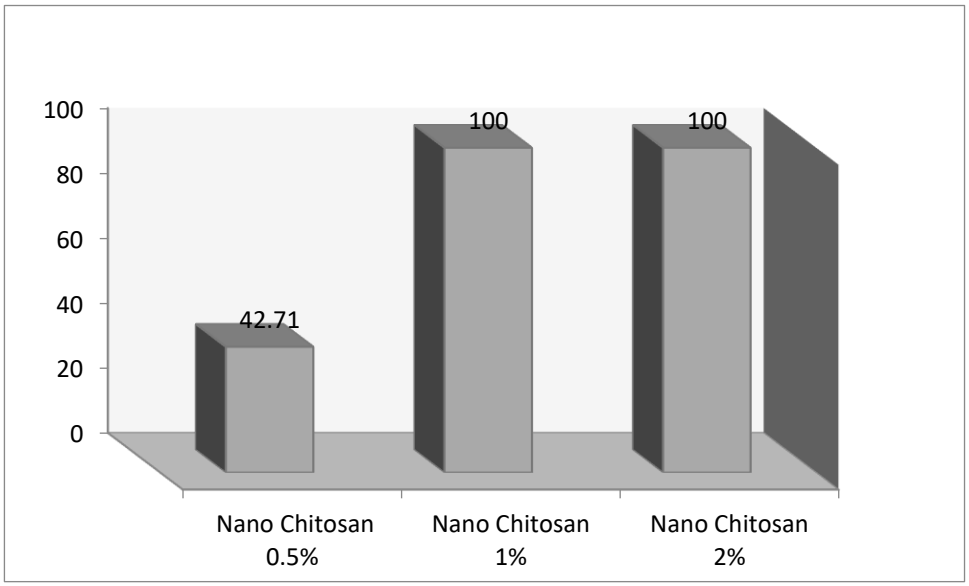

Figure (6). Minimum inhibitory concentration of nanochitosan

4. DISCUSSION

The mechanism of action of antibacterial drugs is a complicated process that differs between G- and G+ bacteria during the course of their activity. This is because the chemical composition of the cell wall and the cell membrane undergoes changes, which causes the mechanism of action to be complex. Previous studies have demonstrated that G- bacteria possess a greater level of antibacterial activity compared to G+ bacteria. However, other studies have demonstrated that G+ bacteria are more susceptible to the effects of the antibacterial agent. Both the mixed hydrophilicity and the negative charge distribution on the surface of bacteria were thought to be the reasons responsible for the various reactions that bacteria exhibited by the researchers. A variety of distinct factors, including the bacterial target (i.e., G- or G+ bacteria) and the development of the bacteria, as well as its concentration, pH, molecular weight, and degree of acetylation, all play a role in determining its activity [15]. By calculation of minimum inhibitory concentration (MIC) and compared with chitosan and chitin activity. The Ch NP compounds exhibited superior antimicrobial activity against all microorganisms in comparison with chitosan. The study is thus a good demonstration of the applicability of chitosan nanoparticles as an effective antimicrobial agent. the smaller size of the chNPs also plays an influential role in the mechanism of antimicrobial activity, which allows them to efficiently pass across the membrane barriers and disrupt the physio-chemical functions of the microorganism [17]. Chitosan is known by a number of other names including polyglusam, deacetylated chitin and poly-D-glucosamine [18].

5. CONCLUSION

The study found that CS-NPs and chitosan are efficient against the microorganisms studied, suggesting that they might be used as antibacterial medicines. These nanoparticles have a good effect on microbial reduction, making them ideal for treating ailments in medicine, agriculture, and the food sector.

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