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*By Universitas Muhammadiyah Sidoarjo*

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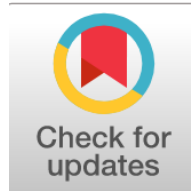
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## **Nano-Chitosan and Ascorbic Acid Synergistically Combat Resistant Staph in Atopic Dermatitis**

*Nano-Chitosan dan Ascorbic Acid Secara Sinergis Memerangi Staph Resisten pada Dermatitis Atopik*

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### **Abstract**

Chitosan, derived from natural sources like fish scales and fungal cell walls, forms nanoparticles (NPs) with notable antimicrobial properties. This study examines the antibacterial effects of ascorbic acid combined with nano-chitosan on *Staphylococcus aureus* isolates from 100 atopic dermatitis (AD) patients. Using the Vitek 2 Compact device for bacterial identification and antibiotic sensitivity testing, we found that nano-chitosan/ascorbic acid composites significantly inhibited the growth of multidrug-resistant *S. aureus*. The antimicrobial activity increased with higher concentrations, highlighting the potential of this natural polymer blend as an effective treatment for AD-related bacterial infections.

#### **Highlights:**

**Effective Inhibition:** Nano-chitosan/ascorbic acid inhibits multidrug-resistant *Staphylococcus aureus*.

**AD Focus:** Targets *S. aureus* in atopic dermatitis patients.

**Green Alternative:** Uses natural, non-toxic antibacterial agents.

**Keywords:** Chitosan nanoparticles, Ascorbic acid, *Staphylococcus aureus*, Atopic dermatitis, Antibacterial treatment

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## Introduction

A common ingredient in medical formulations is chitosan, a naturally occurring polysaccharide [1,2]. Fish scales, the exoskeletons of crustaceans and insects, and the cell walls of fungi are all primarily composed of chitosan, which is generated from chitin. It is a cationic polymer made up of (1-4)-2-amino-2-deoxy- $\beta$ -D-glucan that has garnered greater interest than its base polymer chitin because of its pH sensitivity, biocompatibility, and bioactive properties [3,4]. In recent decades, chitosan nanoparticles (NPs) have drawn much attention as a promising polymeric and bio-based NP. Their potential as nanocarriers to encapsulate medications or active chemicals, transport them to a targeted location, and facilitate a regulated release is enormous. [5]

Depending on the surface modification techniques and preparation procedures employed, the characteristics of chitosan nanoparticles might range significantly, opening up new application opportunities in diverse domains. [6] Numerous reviews have been conducted in the field of medicine, such as the ones found in [7, 8] Aside from the tremendous progress in medicine over the past few decades, scientists have focused on a variety of uses, including wastewater treatment, agriculture, and cosmetics. [9]

Eczema, also known as Atopic Dermatitis (AD) is a prevalent long-term inflammatory skin condition characterized by recurrent *Staphylococcus aureus* infections. With a prevalence of about 20%, this condition mostly affects newborns and children, especially in wealthy nations. [10,11] Skin barrier failure is a result of AD, a multifactorial complex illness with a range of etiologies and consequences. While some cases go away on their own throughout time, others linger into puberty and can progress to become respiratory allergies like allergic rhinitis or asthma. [12]

Atopic dermatitis is a chronic (long-lasting) skin condition that causes skin irritation, redness, and inflammation. Though anyone can contract the disease at any age, it is a prevalent ailment that typically first manifests in childhood. Since atopic dermatitis is not contagious, it cannot be passed from one person to another. [13]

### 1.2 Causes of Atopic Dermatitis

Although the exact etiology of atopic dermatitis is unknown, scientists have discovered that modifications to the skin's protective layer may result in moisture loss. Dry skin may result from this, harming and irritating the skin. [5] According to recent studies, inflammation may be the direct cause of itchy feelings, which then lead to scratching by the patient. This increases the chance of bacterial infection and causes more skin damage.[7]

The colonization of the human nose by *Staphylococcus aureus* is a difficult effect that necessitates adhesion to nasal epithelial cells and the ability to handle host defense and rival resident microorganisms [1]. Public health is seriously threatened by the ongoing and serious problem of *S. aureus* infections acquired in hospitals and communities, which primarily affect youngsters, expectant mothers, and recent postpartum women [2].

## Method

**2.1 Collection of samples:** One hundred patients were sampled between October and February of 2023 from the Department of Dermatology, Venereology, and Allergy at Baqubah Teaching Hospital in the Diyala Governorate. A specialist physician identified the clinical signs of the patient and made the diagnosis of atopic dermatitis. For every patient, two skin swab samples (S) were obtained. Solid media were used to cultivate these 100 samples to promote the growth of *Staphylococcus aureus*. Forty (40%) of the total samples that were taken from one hundred AD patients showed signs of bacterial growth on trypticase soy broth (TSB) agar. The sixty (60%) isolates that were left were cultured and did not exhibit any signs of growth. All isolates that showed signs of growth in growth cultures underwent phenotypic and antibiotic susceptibility testing.

**2.2 Diagnosis of isolated bacteria using the Vitek 2 Compact device:** Use of the Vitek 2 Compact equipment for the diagnosis of isolated bacteria: The Vitek 2 gadget is a crucial step in the diagnosis process for the 20 pure isolates that were identified as *S. aureus* bacteria, a Gram-positive bacterium seen in individuals suffering from severe atopic dermatitis. Confirmatory diagnosis with the Vitek 2 device by utilizing an antibiotic sensitivity test and a phenotypic diagnosis card (GPI card). It takes about eight hours to get the test results. With 99% accuracy, this diagnosis can be made. It is based on several tests to gauge the bacterial type's enzymatic activity and carbon source consumption.

### 2.3 Determine Antibiotics Sensitivity by disc diffusion

1 - The isolate was diluted in (BHIA) and adjusted to  $10^6$  CFU/ml using an ELISA reader. They were grown on Petri dishes equally on each plate and when bacterial growth was determined. Antibiotic susceptibility testing was performed using antibiotic tablets on *Staphylococcus aureus* bacteria according to (Al-Jubouri et al. 2021)

1-The antibiotic tablets used in this study (Doxycycline, Amoxicillin, Cephalexin, Ciprofloxacin, Azithromycin,



Tobramycin, Amikacin...) were placed using sterile metal forceps on the surface of Muller Hinton (which had previously been inoculated with the pathogenic bacteria *Staphylococcus aureus*)

2. The dishes were left to dry for 15 minutes in sterile environmental conditions, and the diameters of the inhibition zone were measured after (24 hours) of anaerobic incubation at (37°C).

### 2.3 Minimum inhibitory Concentration (MIC) of (CHNps) by Method Well Diffusion

1- MIC determination was performed by the method used to clarify the effect of CHNPs dissolved in 100 ml of Ascorbic Acid. The diluted bacterial suspension growing on the BHIA culture medium was planted in dishes, and wells were made evenly distributed in each dish with a diameter of 10 microns. Only Ascorbic Acid was placed in the first well, Chitosan nanomaterial was dissolved in ascorbic acid, at serial concentrations and incubated for 24 hours, and its diameters of inhibition were recorded in milliliters.

2- To determine Bacterial sensitivity testing with chitosan nanoparticles by wells. Nanochitosan dissolved in Serial concentrations of ascorbic acid was placed into wells of equal diameter in dishes previously inoculated with bacteria. 10 microliters of Ascorbic Acid were added to each hole in the inoculated Petri dishes

3- These dishes were left to dry for 15 minutes in sterile environmental conditions, and the diameters of the inhibition zone were measured after (24 hours) of anaerobic incubation at (37°C).

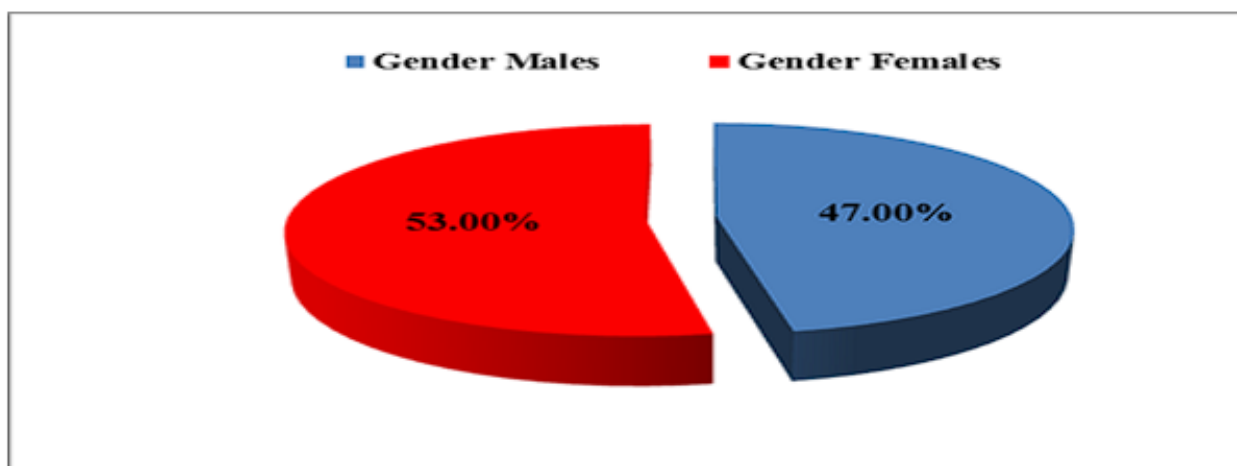
## Result and Discussion

### 3.1 Collection Distribution of study specimens

Atopic dermatitis was found to be highly prevalent in the participants in the current investigation. The study included 100 specimens of chronic patients that were cultured on solid media to facilitate the growth of *Staphylococcus aureus*. Of the 100 specimens from individuals with atopic dermatitis, 40 (40% of the total) showed bacterial growth on trypticase soy broth (TSB) agar. In contrast, the other 60 isolates (60%) did not demonstrate any bacterial growth or other forms of *Staphylococcus aureus*. Tests were conducted on all isolates exhibiting positive growth and antibiotic susceptibility phenotypes. As shown in table (3-1)

Gender	Eczema	Diabetic Foot	Total
Male	47 (47%)	24 (24%)	71 (71%)
Female	43 (43%)	10 (10%)	53 (53%)
Total	66 (66%)	34 (34%)	100 (100%)

**Table 1.** Percentage distribution of samples between male and female patients with eczema.



**Figure 1.** Percentage distribution of samples between male and female patients with eczema and.

The total incidence of atopic dermatitis (eczema) in females was 53 (53%) while the total incidence of atopic dermatitis (eczema) incidence in males was 47%.



### 3.2. Diagnosis of *S. aureus* bacteria using the Vitek 2 Compact device

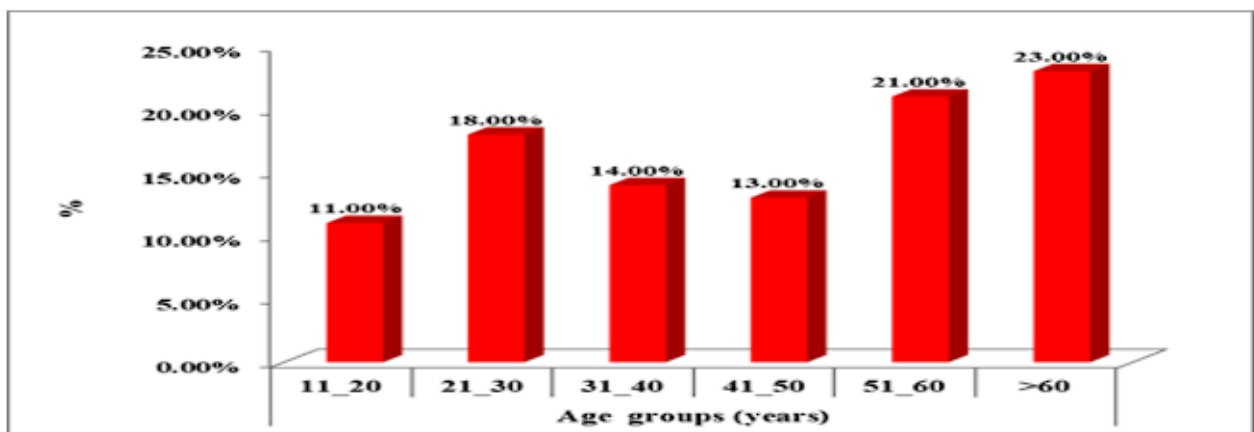
All (40) isolates that showed positive growth, belonging to people with atopic dermatitis and diabetic foot ulcers, were selected for confirmatory diagnosis using the Compact 2 Vitek device, antibiotic susceptibility testing, and phenotypic investigation. (40) isolates were diagnosed as belonging to the *S. aureus* bacteria through the use of a diagnosis card for bacteria positive for the Gram stain in the Vitek 2 device, which gives diagnostic results for the bacteria with an accuracy of up to (99%), these results agree. Many recent studies from Baghdad (Hadi et al., 2023)[14], from America Brandt et al., 2023), [15] and from Diyala (Fajer et al., 2023)[16] , confirm the efficiency of diagnosis using this device.

### 3.3 Distribution of *S. aureus* isolates according to the type of atopic dermatitis and diabetic ulcer

The results of the current study show that the age groups 51-60 and >60 years recorded the highest percentages (21.0% and 23.0%), respectively, compared to the groups 11-20 and 41-50 years, which recorded the lowest percentages (11.0% and 13.0%). respectively for the patients participating in the current study, with statistically significant differences ( $P < 0.05$ ). Female patients with eczema had a higher percentage (53.0%) than Female patients with eczema (47.0%), with statistically significant differences ( $P < 0.05$ ). On the other hand, it was not observed that there were significant differences ( $P > 0.05$ ) between males and females. This also indicates the growth rate of *Staphylococcus aureus* bacteria, as it appeared in 40% of the samples collected from patients with eczema and diabetic ulcer patients in the patients participating in the current study. It was not observed that there are significant differences ( $P > 0.05$ ) as shown in Table (4-2) and Figure (3-2)

		Count	Percent	P value
Gender	Males	47	47.0%	$P > 0.05$
	Females	53	53.0%	
Age groups (years)	11-20	11	11.0%	$P < 0.05^*$
	21-30	18	18.0%	
	31-40	14	14.0%	
	41-50	13	13.0%	
	51-60	21	21.0%	
	>60	23	23.0%	
Eczema	Female	53	53.0%	$P < 0.05^*$
	Male	47	47.0%	
Microbial growth	No	60	60.0%	$P > 0.05$
	<i>Staphylococcus aureus</i>	40	40.0%	

**Table 2.** Distribution of *S. aureus* isolates according to the type of infection in Atopic Dermatitis



**Figure 2.** Distribution of *S. aureus* isolates according to the type of infection in Atopic Dermatitis Ulcers

### 3.4 Antibiotics susceptibility test

Antibiotic susceptibility testing of *Staphylococcus aureus* strains was determined through antimicrobial susceptibility testing performed by the Vitek-2 device to (15) antibiotics. The *S aureus* isolates showed resistance to

(11) antibiotics from different classes and in varying proportions. The highest resistance rate was 38 (95%) to the antibiotic (Tetracycline), then the next. The resistance rate was 37 (92.5%) to the antibiotics (Erythromycin, Clindamycin), while the isolates that showed sensitivity, with a high rate of 33 (82.5%), were to each of the antibiotics (Linezolid Teicoplanin, Vancomycin, Tigecycline). The results were interpreted according to the recommendation of (CLSI, 2020) [17] , as shown in Table (3-4)

Class of Antibiotics	Antibiotics	Code	Sensitive No. (%)	Resistant No. (%)
Pencillines	Benzylopenicillins	(PenG)	8(20%)	32(80%)
	Oxacillin	OX	8(20%)	32 (80%)
Aminoglycoside	Gentamicin	GEN	17(42.5%)	23(57%)
Fluoroquinolones	Ciprofloxacin	CIP	18(45%)	22(55%)
	Moxifloxacin	MOX	22(55%)	18(45%)
Macrolide	Erythromycin	E	3(7.5%)	37(92.5%)
Lincosamine	Clindamycin	CD	3 (7.5%)	37(92.5%)
Oxazolidenosis	Linezolid	LZ	33(82.5%)	7(17.5%)
Glycopeptide	Teicoplanin	TIC	33 (82.5%)	7(17.5%)
	Vancomycin	VAN	33 (82.5%)	7(17.5%)
Tetracycline	Tetracycline	TET	2(5%)	38(95%)
	Tigecycline	TGC	33 (82.5%)	7(17.5%)
Fusidanes	Fusidic Acid	FA	10(25%)	30(75%)
Macrolide	Rifampicin	RIF	11(27.5%)	29(72.5%)
Folate inhibitor	Trimethoprim-Sulfamethoxazole	SXT	29(72.5%)	11(27.5%)

**Table 3.** Aureus resistance test results. S for antibiotics with Vitek 2 device

### 3.5 Effect of Nanochitosan on the growth formation of S. aureus

The current study results show that different concentrations of CNPSA, SA, CNPLA, and LA significantly ( $P < 0.05$ ) on the microbial growth of Staphylococcus aureus bacteria isolated from eczema and diabetic foot ulcers. All materials, CNPSA, SA, recorded the highest inhibitory effect on microbial growth at a concentration of 62.5 ( $0.5828 \pm 0.0360$ ,  $0.6331 \pm 0.0669$ ), compared to a concentration of 2000, which recorded the highest killing effect on microbial growth ( $0.0619 \pm 0.0118$ b,  $0.2244 \pm 0.0771$  a (with significant effect ( $P < 0.05$ )).

Concentrations	MIC Bacterial growth survival %		P value
	CNPSA Mean±SEM	SA Mean±SEM	
2000 (MBC)	$0.0619 \pm 0.0118$ b E	$0.2244 \pm 0.0771$ a B	$P < 0.05^*$
1000	$0.1820 \pm 0.0208$ a D	$0.1525 \pm 0.0279$ a B	$P < 0.05^*$
500	$0.2912 \pm 0.0207$ C	$0.2573 \pm 0.0227$ B	$P > 0.05$
250	$0.3890 \pm 0.0209$ a B	$0.3085 \pm 0.0318$ b B	$P < 0.05^*$
125	$0.4611 \pm 0.0396$ b B	$0.5713 \pm 0.0680$ a A	$P < 0.05^*$
62.5 (MIC)	$0.5828 \pm 0.0360$ a A	$0.6331 \pm 0.0669$ a A	$P < 0.05^*$
31.25	$0.6187 \pm 0.0365$ A	$0.7026 \pm 0.0630$ A	$P > 0.05$
15.6	$0.6609 \pm 0.0369$ A	$0.7217 \pm 0.0639$ A	$P > 0.05$
P value	$P < 0.001^{***}$	$P < 0.001^{***}$	

\*Large letters are compared vertically \*\* Small letters are compared horizontally \*\*\* Different letters indicate significant differences  $P < 0.05$

**Table 4.** The effect of adding CNPSA, SA, at different concentrations on the bacterial growth of Staphylococcus aureus isolated from eczema.

The results of the current study showed a clear effect in the highest inhibition of bacterial growth (MIC) at the concentration of 62.5, so the bacterial growth rate was 0.5828 in the case of the effect of CNPSA solution (chitosan with ascorbic acid) and it was 0.4623, the highest bacterial killing (MBC) at the concentration of 2000, so the lowest bacterial growth rate was 0.0619. In the case of the effect of the CNPSA solution (chitosan with ascorbic acid), it was 0.0443, and as is evident in the cases of killing (MBC) and inhibition (MIC), the use of chitosan dissolved with acetic acid (CNPLA) had a higher effect than the use of the CNPSA solution (chitosan with ascorbic acid). This is due to the thermodynamics of dissolving ascorbic acid, the entropy of dissolution, and the change in

free energy that characterizes ascorbic acid, which increases solubility and increases the permeability of the solution into the bacterial cell, which leads to its inhibition and killing (Sotelo-Boyás et al., 2017) [18].

The results of this study agreed with many studies interested in the effect of chitosan polymers on bacterial killing. They agreed with the study conducted by Algammal et al., 2020 . [19] The killing rate increased with increasing concentration of chitosan during wavelength measurements of bacterial growth turbidity, and the highest reading was at the wavelength (Abs. OD). 620 nm, as it varied between materials with a significant decrease in the growth of the Gram-positive microorganism *S. aureus*. In addition, the antibacterial activity of chitosan was strongly dependent on the concentration, which was discussed in terms of the spatial conformation of the polymer. In the study presented by Alzubaidi (2015) [20] the rate of bacterial inhibition of *S. aureus* at various concentrations was compared to other bacterial isolates that were isolated from wounds and third-degree burns, while the results of this study did not agree with the results reached by Govindan et al. (2023) [21]. The highest inhibitory effect on microbial growth was at a concentration of 250 micrograms/ml

## Statistical analysis

Statistical analysis was conducted using the statistical programs SPSS version 25, Graph pad prism version 6, and Excel 2013. The ordinal data were described in numbers and percentages, and the differences between percentages were compared using the chi-square test. As for the quantitative data, it was described in the form of the arithmetic mean  $\pm$  the standard error of the arithmetic mean, and the comparison between the arithmetic means was made using analysis of variance (ANOVA), in which the Duncan test was used to determine the differences between the arithmetic means, whether they were significant or not. Data were analyzed statistically at a significance level of  $P \leq 0.05$ .

## Conclusion

nano-chitosan/ascorbic acid is an excellent natural polymer that helps to inhibit the growth of *S. aureus* multidrug-resistant bacteria isolated from Atopic Dermatitis AD (eczema) patients at a lower concentration. In increasing the concentration of nano-chitosan/ascorbic acid, the killing of bacteria was observed. Therefore, the present result confirmed that the nano-chitosan/ascorbic acid composites were a more effective nanomaterial for *Staphylococcus aureus*

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