Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 29 (3): 1315 – 1332 (2025)
www.ejabf.journals.ekb.eg

Chitosan and its Derivatives for Removing Antibiotics and Bacteria from Hospital Wastewater and Aquatic Environment

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ARTICLE INFO

Article History:
Received: March 19, 2025
Accepted: May 21, 2025
Online: May 25, 2025

Keywords:
Chitosan,
Salicylaldehyde,
Vanillin,
Antibiotics,
Wastewater,
Hospital

ABSTRACT

Hospitals are a major source of contamination due to the extensive use of antibiotics, leading to the emergence of resistant bacteria. The study was conducted in Kirkuk, Iraq. Wastewater samples were collected from the treatment plant at Azadi Teaching Hospital in Kirkuk, and bacteriological tests were conducted at the Kirkuk Environment Department. Four antibiotics (amoxicillin, azithromycin, ciprofloxacin and metronidazole) were evaluated using HPLC at the Scientific Research Authority - Environment and Water Technology Research Center/Baghdad. Chitosan and its derivatives were extracted from carp scales and were analyzed using (FTIR). Chitosan and its derivatives were used at concentrations of 5, 10, and 15g per liter for water treatment by coagulation. The results showed that salicylaldehyde chitosan was the most effective in removing antibiotics—amoxicillin, azithromycin, ciprofloxacin, and metronidazole—at concentrations of 17.79 ± 8.47, 18.10 ± 7.66, 16.90 ± 8.05, and 6.10 ± 3.53ppm, respectively. Vanillin chitosan removed these antibiotics at concentrations of 21.35 ± 8.45, 19.78 ± 7.12, 19.21 ± 8.20, and 7.95 ± 4.25ppm, while chitosan achieved removal rates of 36.32 ± 10.34, 38.21 ± 7.44, 27.58 ± 6.35, and 12.00 ± 4.35ppm. The treatment efficiency of salicylaldehyde chitosan was 91% for amoxicillin, 84% for azithromycin, 90% for ciprofloxacin, and 90% for metronidazole. The vanillin chitosan derivative achieved 89% efficiency for amoxicillin, 82% for azithromycin, 88% for ciprofloxacin, and 87% for metronidazole. Chitosan alone achieved efficiencies of 81% for amoxicillin, 66% for azithromycin, 83% for ciprofloxacin, and 80% for metronidazole. Regarding bacterial removal, chitosan demonstrated an improved efficiency compared to pretreatment, although the results were not statistically significant.

INTRODUCTION

Hospitals play an essential role in providing health services, but they also produce large amounts of wastewater. In developed countries, each hospital produces between 400 to 1,200 liters of wastewater per bed per day, while in developing countries the production ranges from 200 to 400 liters per capita per day. Wastewater from hospitals is different from that originated from other sources, making it a complex environmental challenge due to its stable characteristics. The size of the hospital also affects the quality and quantity of liquid and solid waste it produces. Hospital wastewater contains a

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variety of contaminants, including antibiotic-resistant bacteria and viruses **(Custodio *et al*., 2021; Rasheed *et al*., 2024)**.

The healthcare sector is environmentally challenged by specialty drug residues released into the environment from hospitals. It is estimated that 90% of oral medications are excreted in wastewater, a major source of pollution. Consumption in developed countries is the biggest contributor to water pollution, with 596 pharmaceuticals registered. Wastewater treatment plants are not designed to fully remove pharmaceuticals, and the efficiency of wastewater treatment plants in removing pharmaceuticals varies, with removal rates ranging from 0 to 97%. Drugs do not degrade easily, and even low concentrations can be alarming, leading to the discharge of drug residues to surface and drinking water **(Stenuick, 2021)**.

Antibiotics are classified as one of the most serious environmental pollutants. The sources of these wastes have been identified, with wastewater from hospitals, agricultural and domestic establishments being major sources of water pollution. Concern about environmental contamination with antibiotics, especially aquatic habitats, is growing on a global scale. Although these drugs are necessary in hospitals, their misuse and disposal leads to large quantities of them being discharged into wastewater. Antibiotic resistance, a public health concern, is exacerbated by the presence of antibiotics in aquatic environments, which may encourage bacterial resistance **(Mahmood *et al*., 2025)**. Studies on antibiotic residues have been conducted in high-income countries, while in low- and middle-income countries they are less frequent, although it is important to record the situation there due to the scarcity of wastewater treatment facilities **(Lien *et al*., 2016; Timofeeva & Bodienkova, 2021)**.

Microbial pathogens are a major health issue associated with water and wastewater. Traditional indicators of fecal contamination include total coliforms, *Escherichia coli*, and *Clostridium perfringens*, which are monitored to assess wastewater treatment processes **(Ajonina *et al*., 2015)**. Antibiotics are ingested and excreted, causing wastewater contamination, thereby negatively impacting soil and water **(Ilurdoz *et al*., 2022)**. A study in the city of Baghdad showed antibiotic levels in drinking water from two water treatment facilities. The results showed the presence of antibiotics in the water, with ciprovoxacin being the most common in the Wahda facility with a concentration of 1,270g/ L, while amoxicillin was not found. In Al-Rasheed facility, ciprofloxacin was detected at a concentration of 1.344g/ L in raw water and 1.312g/ L in fish water, and amoxicillin was the most detectable at a concentration of 1.50g/ L **(Mahmood *et al*., 2019)**. In a study conducted by **Jabbar (2021)** in Baghdad City to color and identify some antibiotics such as tetracycline, ciprofloxacin and gentamicin in water of Tigris River in Baghdad City, the antibiotics showed different levels in water and sediment in dry and wet seasons. Chitosan is a polymer composed of glucosamine and N-acetyl glucosamine, extracted from chitin found in the cell walls of crustaceans, fungi and insects, and can also be extracted from fish scales. Chitosan has unique properties such as biocompatibility and biodegradability, and is non-toxic and hypoallergenic. It also shows great potential as an antibacterial substance **(Putri *et al*., 2021; Pellis *et al*., 2022)**. Chitosan is used in multiple fields such as

pharmaceuticals, cosmetics, and the food industry, where it contributes to food preservation and water purification. Studies have shown its effectiveness in improving the quality of drinking water by removing metals and microbial contaminants, and chitosan is an environmentally friendly sorbent that is effective in treating wastewater contaminants, which enhances its role as a solution to water issues **(Al-Manhel *et al*., 2018)**.

MATERIALS AND METHODS

Study site

Azadi Hospital, which has about 425 beds, is situated in the Al-Iskan area in central Kirkuk. A Japanese business constructed the hospital's own treatment facility in 1985, and it uses the traditional technique known as "activated sludge treatment" **(Jumaa, 2017)**. It is considered a tertiary care facility, treating a variety of patients in departments such as internal medicine, surgery, pediatrics, dermatology, and others **(Majid *et al*., 2023)**. Every day, the hospital produces 200–1,200 liters of wastewater per bed, which needs to be treated. Along with pathogenic microbes, poisonous compounds, and heavy metals, this wastewater also contains pollutants such as detergents, drug residues, and disinfectants. These pollutants endanger the environment by causing pollution **(Sakina *et al.*, 2023)**. Wastewater resulting from human activities in residential, commercial, and agricultural environments, containing microbiological, inorganic, and organic pollutants, is discharged to the sewage network from a variety of sources **(Jumaa, 2017)**.

Bacterial tests were conducted at the Kirkuk Environment Department and antibiotic testing at the Scientific Research Authority - Environment and Water Research and Technology Center (EWRTC) / Baghdad,Iraq.

Chemical extraction of chitosan

Chitosan was extracted based on the method of **Haj *et al*. (2020)**. Carp fish scales were collected from local markets in the city of Kirkuk weighing between (2-3) kilograms, as shown in Fig. (1). Then, the scales were cleaned well from impurities and meat stuck to them with running tap water and were dried at room temperature for 7 days, and milled by a blender brand RAF of Russian origin and sieved by a sieve with 0.07 holes and its weight after grinding ranged about (300 grams) of crusts powder.

Fig. 1. The fish scales

Steps of chitosan extraction

1. Extraction of chitin: After collecting carp fish scales and drying them for 7 days, the fish scales were washed with hot absolute ethanol alcohol with a concentration of 99.9% to remove microbes, germs and strong Odors at a temperature of (50-60℃) by Hote plate and left to dry for (24-48) hours to remove traces of moisture and then it was ground by the previously mentioned blender and converted into powder after sieving it with a sieve or sieve
2. Demineralization : Hydrochloric acid HCl (5%) was added to the peel powder at a temperature of 25℃ and stirred for 24 hours Hote plate and strir of Korean origin to remove CaCo3 and then the powder was filtered with filter papers and washed with distilled water **(Haj *et al*., 2020)**.
3. Protein removal : Sodium hydroxide NaOH (10%) was added to the powder at 60℃ and stirred for 24 hours by the above-mentioned apparatus to remove the protein and the powder was filtered with filter paper and washed with distilled water.

Conversion of chitin to chitosan or deacetylation: The powder was treated with NaOH (50%) at 60℃, stirred for 24 hours, filtered with filter paper, washed with distilled water and obtained chitosan, as shown in Fig. (2). The chitosan was analyzed by FTIR in the laboratory of the Department of Chemistry/College of Science **(Haj *et al*., 2020; Jamal, 2024)**.

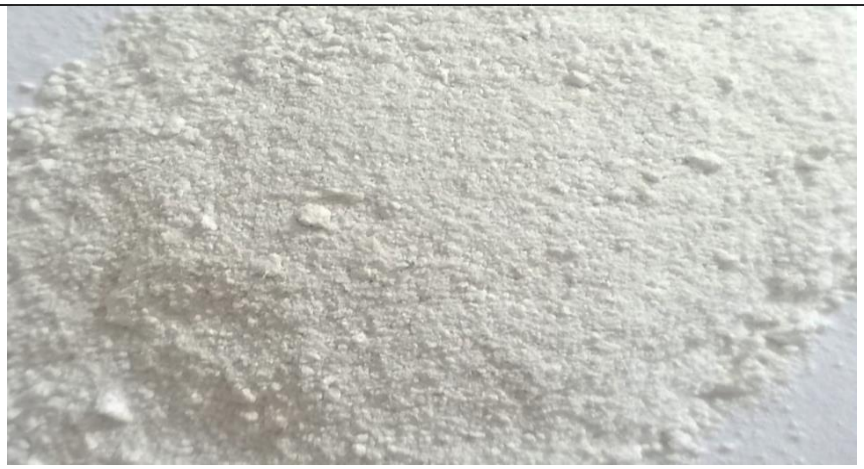

Fig. 2. The prepared chitosan

Steps to extract chitosan derivatives

First derivative Schiff base chitosan and salicylaldehyde

Chitosan Schiff bases were extracted based on the method of **Ismiyarto *et al.* (2021)**. Chitosan (30-40g) was dissolved in 50-75mL of CH3COOH 2% (v/v) and then 0.2mL of salicylaldehyde was added at 50°C in a water bath for 6-12h. The formed product was precipitated with 5% NaOH and filtered through filter paper, and then washed with ethanol and distilled water. The formed product was dried at 60°C, and then was analyzed by FTIR spectroscopy.

Derivative II Schiff base chitosan-phenylene

Chitosan (30-40g) was dissolved in 50-75mL of 2% CH3COOH (v/v), and then 0.35g of vanillin aldehyde was added at 50°C in a water bath for 6-12h. The formed product was precipitated with 5% NaOH and was filtered through filter paper, and then washed with ethanol and distilled water. The formed product was dried at 60°C, and was then analyzed by FTIR spectroscopy.

Bacteriological tests for wastewater

These methods are basic techniques for bacteriological water testing, whether testing for the total number of bacterial colonies or detecting specific types of bacteria such as coliforms and *E. coli*. These tests rely on specialized culture media and specific incubation times, ensuring accurate results that are used to assess the bacteriological integrity of water.

Total plate count for wastewater samples

Plate Count Agar, as described by **Buchbinder *et al.* (1951)**, is recommended by the APHA, FDA, and EPA **(EPA, 2021)** for bacteriological analysis of water. Water samples are screened bacteriologically by counting the number of bacterial colonies that grow on a specific culture medium **(APHA, 2017; WHO, 2017)**.

To prepare the culture medium for colony counting, 7 grams of Plate Count Agar were dissolved in 300mL of sterile distilled water and boiled for 15 minutes. The medium was then sterilized in an autoclave at 151°C and 15 bar for 15 minutes, and subsequently cooled to 45°C. Sterilized Petri dishes were used for plating and were appropriately numbered.

The sample to be tested was mixed thoroughly; then, 1mL was taken and diluted with 9mL of Peptone salt solution. From this dilution, 1mL was placed in the center of each Petri dish. The cooled Plate Count Agar was poured into the dishes and allowed to solidify, ensuring thorough mixing of the sample. All procedures were conducted within a sterilized bacteriological workbench.

Testing water for coliforms

This test uses MacConkey Broth medium to confirm the presence of coliform bacteria in the water to be tested **(APHA, 2017; Baird *et al.*, 2017; WHO, 2017)**. The medium is prepared by mixing 40g of MacConkey Broth with 500mL of distilled water, boiling until fully dissolved, dispensing into glass tubes containing Durham tubes, and sterilizing. After cooling to 40–45°C, 50mL of the water sample is added to the tubes. The caps are closed loosely, and the tubes are incubated at 37°C for 24–48 hours. A yellow color change and gas bubbles in the Durham tube indicate the presence of *E. coli* **(Taher & Saeed, 2023)**.

Most probable number (MPN) test for coliform bacteria in water

The MPN test, recommended by **APHA (2017)**, **Baird *et al.* (2017)** and **WHO (2017)**, is a statistical method used to estimate the concentration of coliform bacteria in water or wastewater. It helps detect potential contamination from human or animal feces **(EPA, 2021)**.

This method involves testing three different sample volumes—10, 1, and 0.1mL—in separate sets of tubes. MacConkey Broth medium is used with Durham tubes to detect gas or acid production. For each volume, 5 tubes are prepared containing the medium and a Durham tube. Samples are added as follows:

- 10mL of sample into five tubes
- 1mL into another five tubes
- 0.1mL into another five tubes

Antibiotics investigated in this study

- Amoxicillin
- Azithromycin
- Ciprofloxacin
- Metronidazole

Extraction of antibiotic samples from wastewater

SCX-type solid-phase extraction (SPE) cartridges were pre-conditioned with 10mL of methanol, followed by 10mL of ultrapure distilled water. The pH of the wastewater samples was adjusted to 3 using 0.5 N HCl, according to the method reported in **Feitosa *et al.* (2007)**. SPE cartridges were loaded with 100mL of wastewater sample prepared with 5% (w/v) $Na_2EDTA{\cdot}2H_2O$ to emulsify metal residues. Samples were passed through the cartridges at a constant flow rate of 1.2 mL/min. Retained compounds were eluted with 6mL of methanol, concentrated to dryness using a rotary evaporator at 40°C, and re-dissolved in 2mL of ultrapure acetonitrile. All steps were performed in triplicate to ensure accuracy **(Opriş *et al.*, 2013)**.

HPLC analysis

The test was conducted at the Scientific Research Authority – Environmental and Water Research and Technology Center (EWRTC) in Baghdad. An HPLC SYKAM system was used with a C18 column at 30°C and gradient elution. Eluents included ultrapure water with formic acid (pH 3) and acetonitrile. The gradient program involved reducing eluent A from 90 to 30%, then to 20%, followed by a rapid increase back to 90%, with 5 minutes of column equilibration. The injection volume was 100µL, and the flow rate was 1.0 mL/min. Detection was performed at 272nm **(Opriş *et al.*, 2013)**.

Statistical analysis

All statistical analyses were performed using IBM SPSS Statistics version 28. Data were expressed as mean ± standard deviation (SD). Two-way ANOVA was used to evaluate differences in physical, chemical, microbiological, and pharmaceutical parameters among wastewater samples from Azadi Teaching Hospital. Tukey's post hoc test was applied for pairwise comparisons. Graphs were generated using Excel 2021. A *P*-value of less than 0.05 was considered statistically significant. Uppercase and lowercase letters in the tables indicate significant differences between hospital means for each parameter **(Field, 2024)**.

RESULTS AND DISCUSSION

Wastewater treatment steps

Azadi Teaching Hospital was selected as the model site for applying the treatment procedures. Wastewater samples (1 liter each) were collected early in the morning. Chitosan and its derivatives were tested at concentrations of 5, 10, and 15 g/L. After initial filtration, chitosan was added to the samples at the specified concentrations. The mixtures were stirred for 5 to 10 minutes and then left to settle for 48 hours. Following this, the sediments and residues were filtered out, and the treated water was tested for antibiotics, bacteria, BOD, and COD. These same procedures were applied using the chitosan derivatives, including salicylaldehyde chitosan and vanillin chitosan

(Rockson-Itiveh *et al*., 2024; Ibrahim *et al*., 2025) with three independent replicates. Fig. (3) shows untreated water and after treatment with chitosan.

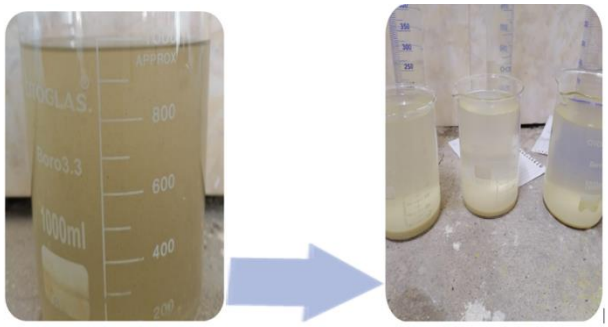

Fig. 3. Wastewater treatment by chitosan

Table 1. Concentrations of chitosan, chitosan salicylaldehyde and vanillin chitosan and their effect on total plate count

T.P.C (Mean ± SD)	Chitosan (C)	Chitosan salicylaldehyde (CS)	Chitosan vanillin (CV)	Univariate *P*-value
Before	363.50 ± 26.44			
C. 5 gm	512.00 ± 6.56 a	477.33 ± 5.03 a	585.67 ± 8.33 a	
C. 10 gm	257.33 ± 11.24 b	379.67 ± 5.51 b	405.33 ± 7.64 b	
C. 15 gm	119.67 ± 9.50 c	202.67 ± 5.03 c	244.00 ± 5.57 c	
Total	296.33 ± 172.57	353.22 ± 120.66	411.67 ± 148.16	0.277 NS
***P*-value**	0.465 ns	0.872 ns	0.541 ns	

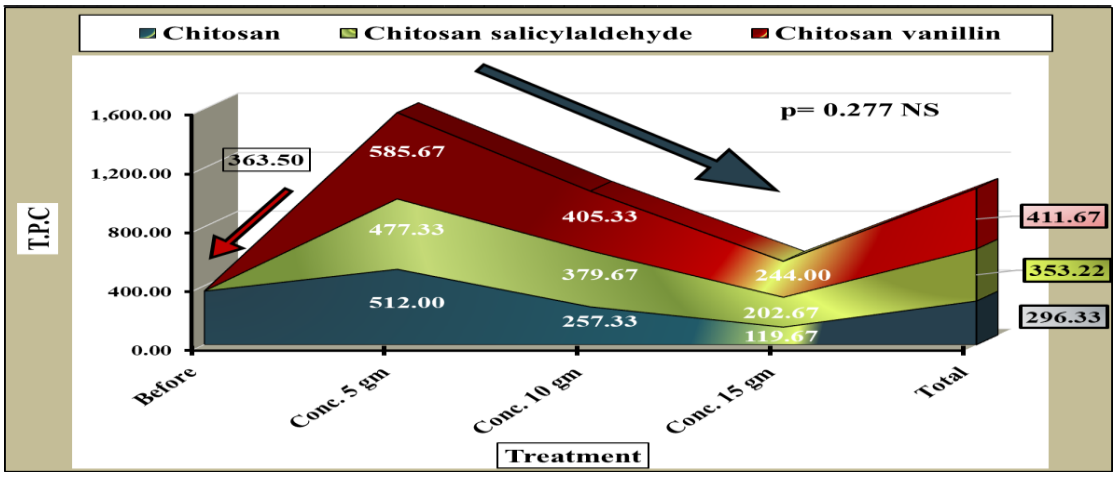

Fig. 4. Concentrations of chitosan, chitosan salicylaldehyde and vanillin chitosan and the effect on T.P.C

Table 2. Concentrations of chitosan, chitosan salicylaldehyde and vanillin chitosan and the effect on total coli form

M.P.N. of Total coliform /100 ml	Chitosan (C)	Chitosan salicylaldehyde (CS)	Chitosan vanillin (CV)	Univariate *P*-value
Before	1,602,500.00 ± 12,583.06			
C. 5 gm	51,910.00 ± 36.06 a	57,650.00 ± 100.00 a	59,694.67 ± 6.81 a	
C. 10 gm	26,673.33 ± 25.17 b	39,226.67 ± 15.28 b	41,195.00 ± 5.57 b	
C. 15 gm	12,783.33 ± 160.73 c	27,263.67 ± 17.04 c	26,592.33 ± 6.81 c	
Total	30,455.56 ± 17,178.38	41,380.11 ± 13,256.52	42,494.00 ± 14,366.80	0.193 NS
***P*-value**	0.001*	0.001*	0.001*	

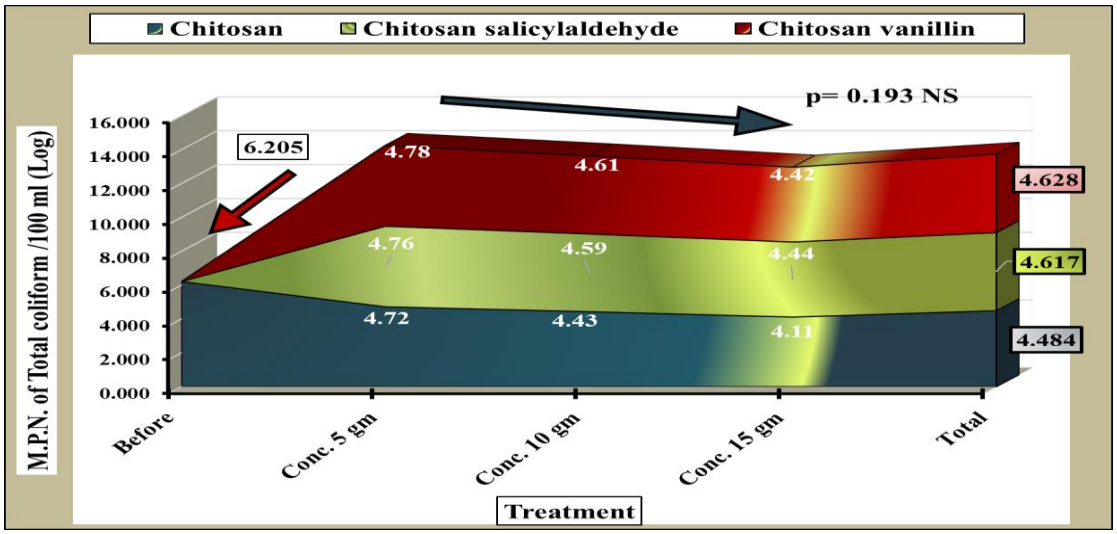

Fig. 5. Concentrations of chitosan, chitosan salicylaldehyde and vanillin chitosan and the effect on total coliform

Table 3. Concentrations of chitosan, chitosan salicylaldehyde and vanillin chitosan and the effect on fecal coliform

M.P.N. of Fecal coli/100 ml	Chitosan (C)	Chitosan salicylaldehyde (CS)	Chitosan vanillin (CV)	Univariate *P*-value
Before	1,622,750.00 ± 37,924.27			
C. 5 gm	2,353.33 ± 41.63 a	2,275.33 ± 5.69 a	2,276.00 ± 4.00 a	
C. 10 gm	1,126.67 ± 64.29 b	986.00 ± 4.58 b	2,152.33 ± 7.51 b	
C. 15 gm	491.00 ± 8.54 c	916.67 ± 9.71 c	931.33 ± 3.51 c	
Total	1,323.67 ± 820.74	1,392.67 ± 662.71	1,786.56 ± 643.66	0.348 NS
***P*-value**	0.001*	0.001*	0.001*	

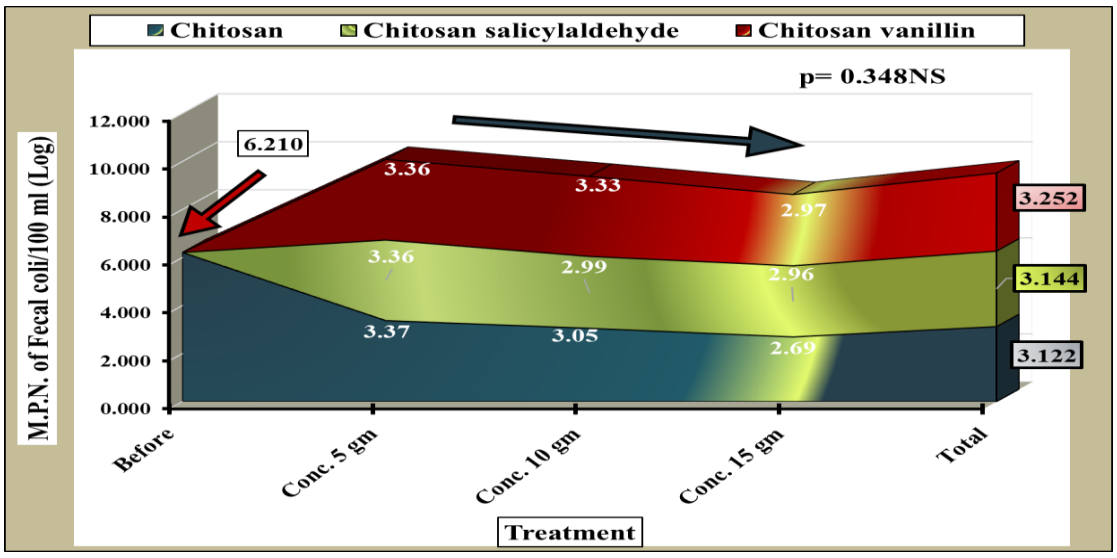

Fig. 6. Concentrations of chitosan, chitosan salicylaldehyde and vanillin chitosan and the effect on faecal coliform

Table 4. Concentrations of chitosan, chitosan salicylaldehyde and vanillin chitosan and the effect on *E. coli*

M.P.N. of E.coli /100 ml	Chitosan (C)	Chitosan salicylaldehyde (CS)	Chitosan vanillin (CV)	Univariate *P*-value
Before	1,595,750.00 ± 7,228.42			
C. 5 gm	17,263.33 ± 55.08 a	19,241.00 ± 13.75 a	23,055.00 ± 39.05 a	
C. 10 gm	8,888.67 ± 13.05 b	13,071.33 ± 7.64 b	13,989.33 ± 26.10 b	
C. 15 gm	4,295.67 ± 10.26 c	8,745.00 ± 561.36 c	9,725.33 ± 25.58 c	
Total	10,149.22 ± 5,694.27	13,685.78 ± 4,576.83	15,589.89 ± 5,895.48	0.118 NS
***P*-value**	0.001*	0.001*	0.001*	

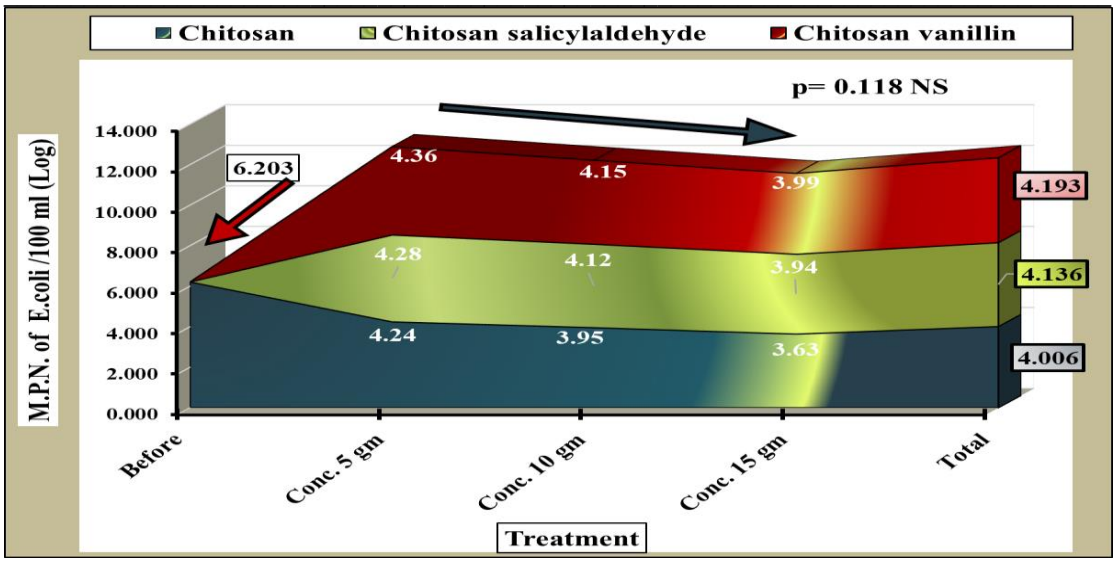

Fig. 7. Concentrations of chitosan, chitosan salicylaldehyde and vanillin chitosan and the effect on *E. coli*

Table 5. Concentrations of chitosan, chitosan salicylaldehyde and vanillin chitosan and the effect on amoxillin

Amoxicillin (ppm)	Chitosan (C)	Chitosan salicylaldehyde (CS)	Chitosan vanillin (CV)	Univariate *P*-value
Before	198.62 ± 0.54			
C. 5 gmm	49.02 ± 0.06 a	28.35 ± 0.03 a	32.28 ± 0.06 a	
C. 10 gm	34.62 ± 0.05 b	16.01 ± 0.05 b	18.22 ± 0.03 b	
C. 15 gm	25.32 ± 0.04 c	9.02 ± 0.04 c	13.54 ± 0.05 c	
Total	36.32 ± 10.34 A	17.79 ± 8.47 B	21.35 ± 8.45 B	0.001*
***P*-value**	0.001*	0.001*	0.001*	

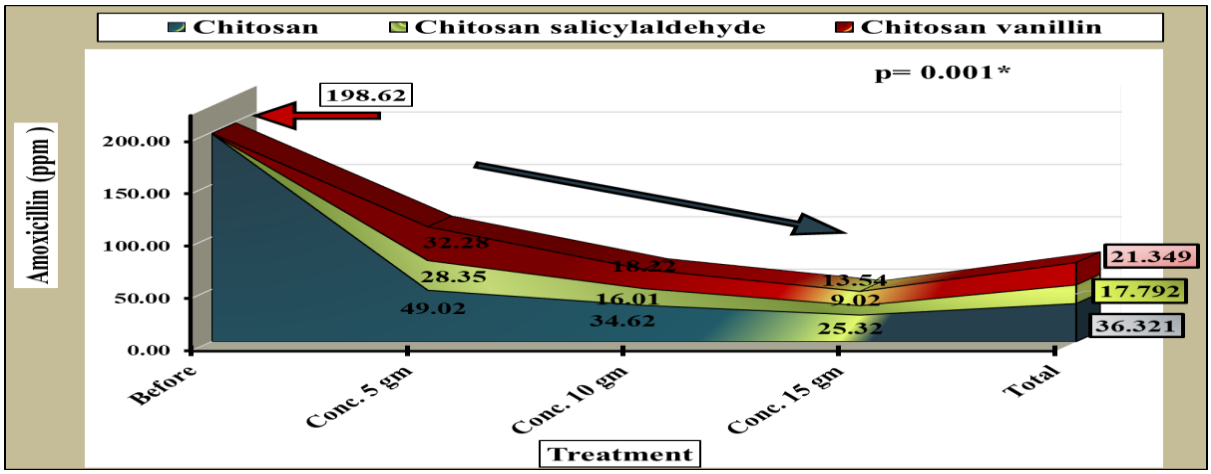

Fig. 8. Concentrations of chitosan, chitosan salicylaldehyde and vanillin chitosan and its effect on Amoxillin

Table 6. Concentrations of chitosan, chitosan salicylaldehyde and vanillin chitosan and the effect on azithromycin

Azithromycin (ppm)	Chitosan (C)	Chitosan salicylaldehyde (CS)	Chitosan vanillin (CV)	Univariate *P*-value
Before	112.15 ± 0.48			
C. 5 gm	45.90 ± 0.03 a	27.50 ± 0.04 a	28.71 ± 0.05 a	
C. 10 gm	39.81 ± 0.07 b	16.88 ± 0.05 b	18.11 ± 0.02 b	
C. 15 gm	28.93 ± 0.05 c	9.93 ± 0.04 c	12.52 ± 0.06 c	
Total	38.21 ± 7.44 A	18.10 ± 7.66 B	19.78 ± 7.12 B	0.0001*
***P*-value**	0.001*	0.001*	0.001*	

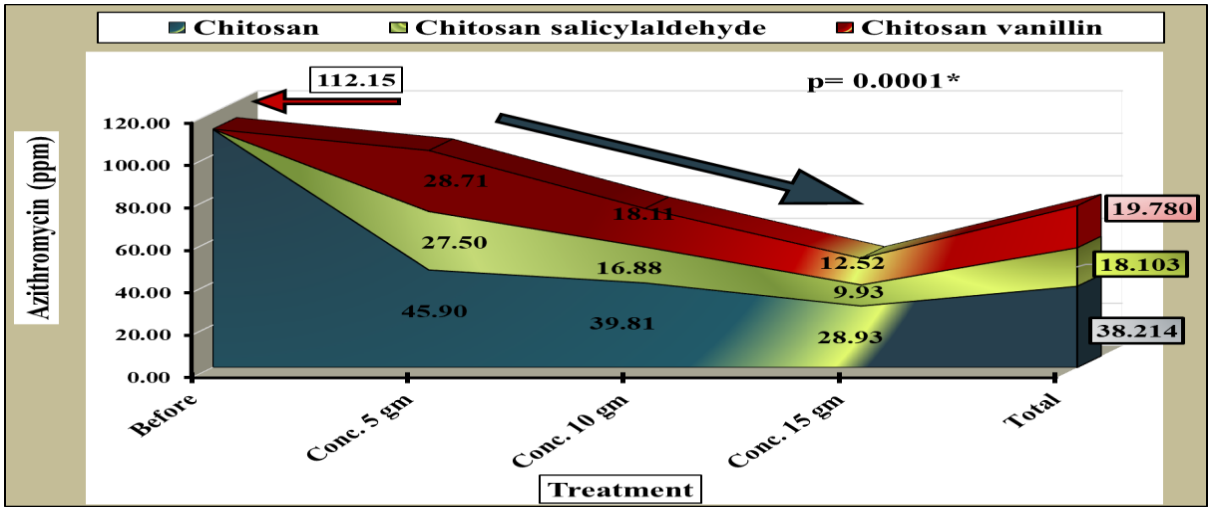

Fig. 9. Concentrations of chitosan, chitosan salicylaldehyde and vanillin chitosan and the effect on Azithromycin

Table 7. Concentrations of chitosan, chitosan salicylaldehyde and vanillin chitosan and the effect on ciprofloxacin

Ciprofloxacin (ppm)	Chitosan (C)	Chitosan salicylaldehyde (CS)	Chitosan vanillin (CV)	Univariate *P*-value
Before	166.30 ± 0.78			
C. 5 gm	35.00 ± 0.06 a	26.24 ± 0.04 a	28.31 ± 0.02 a	
C. 10 gm	27.43 ± 0.05 b	16.82 ± 0.05 b	19.91 ± 0.02 b	
C. 15 gm	20.33 ± 0.06 c	7.65 ± 0.04 c	9.41 ± 0.03 c	
Total	27.58 ± 6.35 A	16.90 ± 8.05 B	19.21 ± 8.20 B	0.016*
***P*-value**	0.001*	0.001*	0.001*	

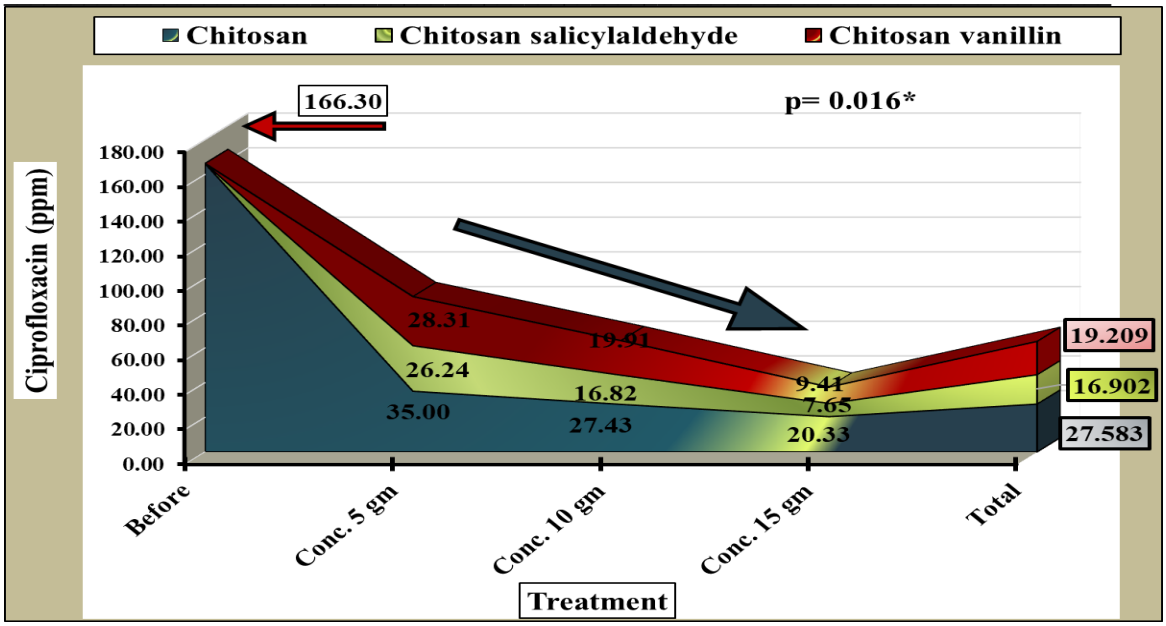

Fig. 10. Concentrations of chitosan, chitosan salicylaldehyde and vanillin chitosan and the effect on ciprofloxacin

Table 8. Concentrations of chitosan, chitosan salicylaldehyde and vanillin chitosan and the effect on metronedazole

Metronidazole (ppm)	Chitosan (C)	Chitosan salicylaldehyde (CS)	Chitosan vanillin (CV)	Univariate *P*-value
Before	60.48 ± 0.25			
C. 5 gm	17.43 ± 0.06 a	10.42 ± 0.03 a	12.92 ± 0.04 a	
C. 10 gm	11.03 ± 0.06 b	5.54 ± 0.05 b	7.82 ± 0.03 b	
C. 15 gm	7.54 ± 0.05 c	2.33 ± 0.25 c	3.11 ± 0.02 c	
Total	12.00 ± 4.35 A	6.10 ± 3.53 B	7.95 ± 4.25 B	0.016*
***P*-value**	0.001*	0.001*	0.001*	

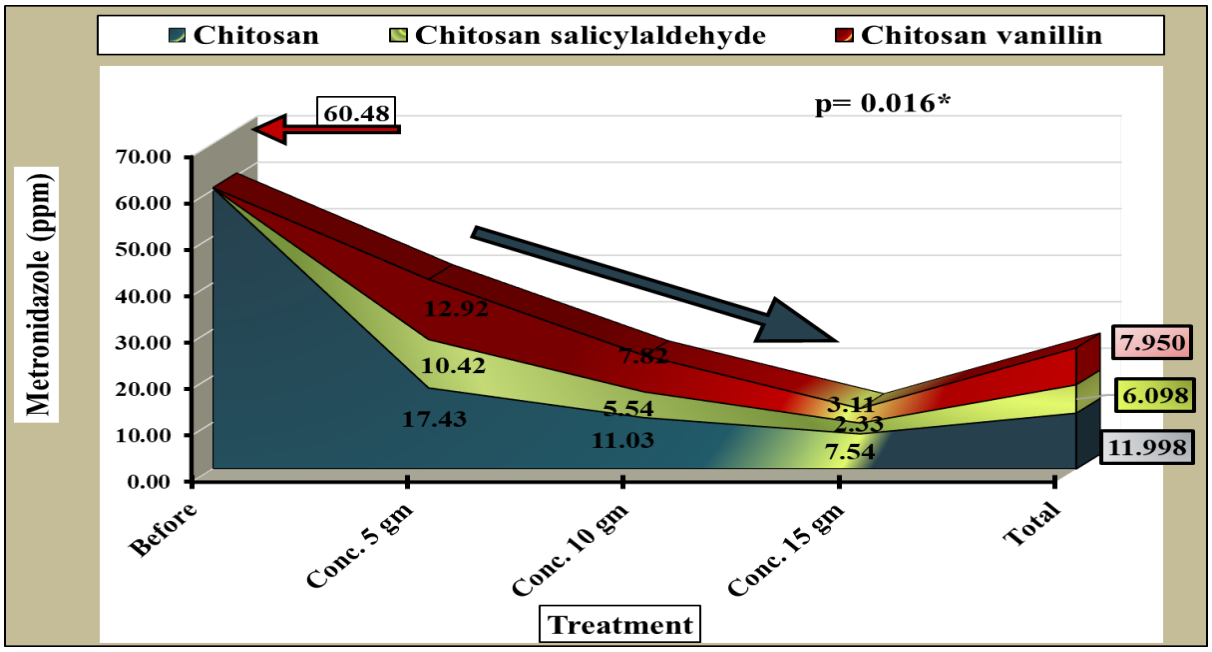

Fig. 11. Concentrations of chitosan, chitosan salicylaldehyde and vanillin chitosan and the effect on metronedazole

The results of the study showed that treatment with chitosan and its derivatives led to a reduction in total coliforms, fecal coliforms, *E. coli*, and total plate count (TPC), with effectiveness increasing as the concentration of the substance increased. The highest bacterial counts were recorded at a concentration of 5g/ L, while the lowest were observed at 15 g/L across all treatments. As shown in Tables (1–4) and Figs. (4–7), there were clear differences among the various concentrations of each substance. The antibacterial effect was most pronounced with chitosan (C), consistent with findings by **Darweesh *et al.* (2020)**, who evaluated chitosan's efficacy against several bacterial types by observing a decrease in total bacterial count. Their study demonstrated that using 0.6g of chitosan with 30 minutes of contact time and 250 rpm agitation eliminated 99.98% of bacterial types, including *E. coli*. These results confirm the potential of chitosan for disinfecting secondary-treated wastewater.

A related study by **Sahaya Victoria *et al.* (2024)**, conducted on the Kanyakumari coast, also supports these findings. It showed that fecal bacteria levels in the control sample reached 6.42×10^{8} CFU/100 mL, while no bacterial growth was detected in the chitosan-treated sample, demonstrating chitosan's effectiveness in inhibiting pathogenic bacterial growth.

Following chitosan in effectiveness was chitosan salicylaldehyde (CS), then chitosan vanillin (CV). This aligns with the results of **Ismiyarto *et al.* (2021)**, who investigated the antimicrobial activity of chitosan combined with Schiff bases of salicylaldehyde and vanillin. They reported better inhibition against *Staphylococcus aureus* (Gram-positive) than *E. coli* (Gram-negative), with 69% inhibition for *S. aureus* and only 7% for *E. coli*. In another study, salicylaldehyde (SA) was used as a crosslinking agent with chitosan to form a hydrogel membrane containing titanium

dioxide nanoparticles via a casting method. These membranes showed effective antimicrobial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*, completely eliminating the bacteria studied **(Montaser *et al.*, 2019)**.

Treatment with chitosan and its derivatives also led to a statistically significant ($P < 0.05$) reduction in the concentrations of the antibiotics amoxicillin, azithromycin, ciprofloxacin, and metronidazole. As detailed in Tables (5–8) and Figs. (8–11), the type of derivative used significantly influenced removal efficiency. Chitosan salicylaldehyde resulted in the lowest residual antibiotic concentrations. This finding is in line with the study by **Mehrabadi and Faghihian (2018)**, which reported enhanced photodegradation efficiency of atenolol using a TiO_2 photocatalyst immobilized on salicylaldehyde-modified supports. Degradation efficiency reached 60% within 60 minutes under visible light, especially when SN-MIL-101(Cr) was used.

Supporting this, **dos Santos Cardoso and Vitali (2021)** evaluated the effectiveness of a biosorbent made from vanillin-modified chitosan (CTSV) for removing emerging pollutants such as isoniazid, cortisol, bisphenol A, 17α-ethinyl estradiol, and triclosan. Their results showed optimal performance at 0.7 mol/L NaCl, confirming CTSV's promise as a low-cost, high-efficiency adsorbent.

Chitosan also proved effective in other studies. **Dinarvand and Moradi (2025)** reported successful removal of various pharmaceuticals—including antibiotics, analgesics, and hormones—using chitosan-based nanocomposites. Furthermore, **Mohammed *et al.* (2023)** demonstrated that copper ferrite ($CuFe_2O_4$) immobilized on chitosan ($CuFe_2O_4$@Chitosan) effectively removed antibiotics such as ciprofloxacin (94.6%), ampicillin (92%), and erythromycin (90.3%) from water, highlighting its potential as a powerful photocatalyst for antibiotic removal.

CONCLUSION

In our current study, pre-treatment wastewater samples were taken from the treatment plant of Azadi Teaching Hospital in Kirkuk. The study showed that chitosan and its derivatives salicylaldehyde and vanillin chitosan are efficient in removing antibiotics, and the results showed a significant improvement in the proportions of coliform, fecal and total bacteria and total coliforms. However, this efficiency is low and was not statistically significant, which indicates that the coagulation method does not help in reducing bacteria, but does not treat them, so it is recommended to use modern methods based on the use of chitosan, salicylaldehyde and vanillin with modern nanomethods or other methods.

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