ISSN: 0067-2904

Antimicrobial, Antibiofilm, and Antioxidant Activity of Bacterial Cellulose and Bacterial Nanocellulose from Vinegar's Mother Pellicles

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Received: 15/5/2024 Accepted: 5/7/2024 Published: 30/6/2025

Abstract

Recent research indicates that bacterial cellulose (BC) and bacterial nanocellulose (BNC) exhibit promising adsorptive properties for antimicrobial agents, owing to their complex network structure, high surface area, and substantial porosity. The aim of this study was to analyze the impact of the antimicrobial, antibiofilm, activity of BC and BNC on different bacterial isolates, were collected from wounds and burn wound patients. Also, a study examined the antioxidant activity. In this study, (BC) and (BNC) was produced from vingar mothers. The Pathogenic bacterial isolates were collected from wounds and burn wound patients of different age groups. The antibiotic susceptibility testing by disk diffusion method and Vitek 2 compact system method. The antimicrobial activity of BC and BNC on different bacterial isolates by the Resazurin-based 96-well plate microdilution method. Detection of biofilm formation and Inhibition of Biofilm Formation was quantified using the 96-well tissue culture plate method (TCP). Finally, the DPPH radical scavenging assay was used to investigate the ability of (BC) and (BNC) to scavenge free radicals. Seventy bacterial isolates were collected from wounds and burn wound patients of different age groups. It belongs to five species: *Citrobacter freundii*, ten isolates; *Escherichia coli*, 17 isolates; *Klebsiella pneumonia*, 16 isolates; *Pseudomonas aeruginosa*, 15 isolates and *Staphylococcus aureus*, 20 isolates. The Antibiotic susceptibility testing by disk diffusion method and Vitek 2 compact system method showed that most bacterial isolates were resistant to the antibiotics used. The antimicrobial activity of BC and BNC on different bacterial isolates by the Resazurin-based 96-well plate microdilution method showed different results in tests bacteria using the minimum inhibitory concentration (MIC). The study found that 60 out of the 70 bacterial isolates were able to produce biofilm, accounting for 85.71% of isolates, while 10 isolates (14.28%) did not produce biofilm. The effect of the BC and BNC on the biofilm formation using SUB-MIC 1 shows that BC and BNC prevent the biofilm formation in 17 (78%) and 25 (100%) isolates. Meanwhile, SUB-MIC 2 of BC and BNC prevent biofilm formation in 10 (42%) and 23 (80%) isolates. The results show that the % PDDH Radical Scavenging Activity for BC was (10.316 and 75.122) when the concentrations were (1.95 and 1000) µg/ml, respectively. The % PDDH Radical Scavenging Activity for BNC was (14.806 and 98.837) when the concentrations were (1.95 and 1000) µg/ml respectively. The % PDDH Radical Scavenging Activity for AA was (16.213 and 98.940) when the concentrations were (1.95 and 1000) µg/ml respectively. The bacterial cellulose and bacterial nanocellulose are characterized by their antimicrobial, antibiofilm, and antioxidant properties, which allow for a wide range of medical, industrial, and environmental applications.

Keywords: antimicrobial, antibiofilm, antioxidant, bacterial cellulose, bacterial nanocellulose.

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النشاط المضاد للميكروبات والأغشية الحيوية ومضادات الأكسدة للسليلوز البكتيري والنانوسليلوز البكتيري من جليدة ام الخل العراقي

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الخلاصة

تشير الأبحاث الحديثة إلى أن السليلوز البكتيري (BC) والنانوسليلوز البكتيري (BNC) يظهران خصائص ادمصاصية واعدة للعوامل المضادة للميكروبات، وذلك بسبب تركيبة شبكتها المعقدة ومساحة سطحها العالية ومساميتها الكبيرة. الهدف من هذه الدراسة هو تحليل تأثير مضادات الميكروبات ومضادات الفيروسات ونشاط السليلوز البكتيري والنانوسليلوز البكتيري على العزلات البكتيرية المختلفة التي تم جمعها من الجروح ومرضى جروح الحروق. أيضا، بحثت دراسة نشاط مضادات الأكسدة. في هذه الدراسة تم إنتاج السليلوز البكتيري والنانوسليلوز البكتيري من جليدة أم الخل. تم جمع العزلات البكتيرية المسببة للأمراض من مرضى الجروح وجروح الحروق من مختلف الفئات العمرية. اختبار الحساسية للمضادات الحيوية بطريقة الانتشار على القرص وطريقة نظام فايتك 2. النشاط المضاد للميكروبات للسليلوز البكتيري والنانوسليلوز البكتيري على عزلات بكتيرية مختلفة بواسطة طريقة صفيحة التخفيف الدقيق المكونة من 96 حفرة من الريسازورين. تم الكشف عن تكوين الأغشية الحيوية وتثبيط تكوين الأغشية الحيوية باستخدام طريقة صفيحة زراعة الأنسجة ذات 96 حفرة. وأخيرا، تم استخدام مقايسة الكسح الجذري للتحقق في قدرة السليلوز البكتيري والنانوسليلوز البكتيري على كسح الجذور الحرة. تم جمع سبعين عزلة بكتيرية من مرضى الجروح وجروح الحروق من مختلف الفئات العمرية. وهي تعود إلى خمسة أنواع: من *Citrobacter freundii* بكتريا عشر عزلات، من *Escherichia coli* 17 عزلة، من *Klebsiella pneumonia* 16 عزلة، من *Pseudomonas aeruginosa* 15 عزلة ومن *Staphylococcus aureus* 20 عزلة. أظهر اختبار الحساسية للمضادات الحيوية بطريقة الانتشار على القرص وطريقة نظام فايتك 2 أن معظم العزلات البكتيرية كانت مقاومة للمضادات الحيوية المستخدمة. أظهر النشاط المضاد للميكروبات للسليلوز البكتيري والنانوسليلوز البكتيري على عزلات بكتيرية مختلفة بواسطة طريقة التخفيف الدقيق للصفيحة المكونة من 96 حفرة اعتمادا على الريسازورين نتائج مختلفة في البكتيريا المختبرة باستخدام التركيز المثبط الادنى (MIC). ووجدت الدراسة أن 60 عزلة بكتيرية من أصل 70 كانت قادرة على إنتاج الأغشية الحيوية، وهو ما يمثل 85.71% من العزلات، في حين أن 10 عزلات (14.28%) لم تنتج الأغشية الحيوية. يوضح تأثير للسليلوز البكتيري والنانوسليلوز البكتيري على تكوين الغشاء الحيوي باستخدام التركيز المثبط تحت الادنى 1 أن السليلوز البكتيري والنانوسليلوز البكتيري يمنعان تكوين الغشاء الحيوي في 17 عزلة (78%) و25 عزلة (100%). وفي الوقت نفسه، يمنع التركيز المثبط تحت الادنى 2 من السليلوز البكتيري والنانوسليلوز البكتيري تكوين الأغشية الحيوية في 10 عزلات (42%) و23 عزلة (80%). أظهرت النتائج أن نشاط الكسح الجذري للسليلز البكتيري كان (10.316 و 75.122)% عندما كان التركيزان (1.95 و 1000) ميكروغرام/مل، على التوالي. وكان نشاط الكسح الجذري للنانوسليلوز البكتيري (14.806 و 98.837)% عندما كان التركيز (1.95 و 1000) ميكروغرام/مل على التوالي. كان نشاط الكسح الجذري لحامض الخليك (16.213 و 98.940) عندما كان التركيز (1.95 و 1000) ميكروغرام/مل على التوالي. يتميز السليلوز البكتيري والنانوسليلوز البكتيري بخصائصهما المضادة للميكروبات والفيروسات ومضادات الأكسدة، مما يسمح بمجموعة واسعة من التطبيقات الطبية والصناعية والبيئية.

1. Introduction

Cellulose, the most abundant biological macromolecule on Earth, is a biopolymer consisting of D-glucose units linked by β(1→4) glycosidic bonds. This linear structure contributes to its insolubility in water [1]. Hydrogen bonds form between the hydroxyl groups of various chains connected by glucose, creating a water-impermeable structure [2]. Marine algae and prokaryotic microbes create cellulose that lacks lignin and hemicellulose, possessing exceptional purity, notable water retention ability, strong mechanical properties, porosity, biocompatibility, and high crystallinity [3]. Cellulose is synthesized by several life forms, from bacteria to plants, and acquired by top-down and bottom-up methods [4] and [5].
Cellulose is available from bacterial sources in a variety of Gram-negative and Gram-positive species as well as plant origin such as wood, cotton and bamboo [6]. Species from the genera including; *Achromobacter, Aerobacter, Acetobacter*, *Komagataeibacter*, *Alcaligenes*, *Pseudomonas Sarcina* and *Rhizobium* are commonly used. *Among the species, Komagataeibacter xylinus, Komagataeibacter hansenii, and Acetobacter pasteurianus are used in a large scale due to their maximum production*. The properties and structure of cellulose will be influenced by the type of bacterium that is used [7]. This particular type of cellulose is also known as bacterial cellulose (BC) or microbial cellulose (MC). Some of the well-established acetic acid bacteria (AAB) genera that are recognized cellulose producers include Acetobacter, Komagataeibacter, and Gluconacetobacter. BC is characterized by having Iα and Iβ allomorphs which distinguishes the bacterial type of cellulose from that of the plant type. Iα allomorph is more predominant in BC structure as compared to the plant cellulose where Iβ allomorph is reported to be found in a large extent. In this case, it was confirmed the Cellulose Iα chains aligned in a dense manner with the size of the order of nanometers [8]. Acetic acid bacteria synthesize two types of cellulose: The native cellulose which is predominant in plant cell walls is cellulosic I, which has a ribbon-like structure and the regenerated cellulose which is cellulose II having an amorphous structure and being more stable than cellulose I. There are distinctions between the synthesis of cellulose I and cellulose II on the type of cellulose formed and the area where is synthesized and it occurs outside the cytoplasmic membrane [7].

The characteristics of a BC are dependent on its nanostructure; BC's broader contour is a result of the type of culturing and the bioreactor used. I often get this one in the hydrogel and then the other form could be in the freeze-drying form. It is essential that the composition of the medium be adjusted because the microorganisms are able to modify protein synthesis at a cellular level and the shape of the cell as well.

It is found that bacterial cellulose is finer than plant cellulose, whereas BC fibers are one hundred times smaller. This 3D network is created by weaved nanofibers making the surface-area-to-volume ratio higher, thus enhancing the compatibility with neighbouring components. It is extremely biocompatible le [9]. The fibrils slightly build up a three dimensional porous structure that has a diametric range of about 10-100 nanometres. The material also delivers excellent mechanical properties which include high degree of polymerization and crystallinity that is estimated to be around 90 percent, and water absorbent capacity. BC is a more environmentally-friendly alternative to the chemical process involved in extracting plant cellulose. The chemical process, which typically utilizes CS2 and heavy metals, can have significant ecological burdens associated with it [10].

It is also possible to produce cellulose using a non-living culture of bacteria and yeast (SCOBY). [11]. Cellulose biopolymer synthesized during the interaction between acetic acid bacteria and yeasts is a symbiotic culture of bacteria and yeast. Kombucha is a SCOBY Symbiotic culture of bacteria and yeast that may form during the process of fermentation [12]. This study aimed at developing new cost-efficient bacterial cellulose (BC) and bacterial

nanocellulose (BNS) by the mother pellicle of Iraqi vinegar for the purposes of ascertaining their antimicrobial, antiviral, and antioxidant properties, which are distinct from those of plant cellulose.

2. Materials and Methods

2.1 Culture Conditions, Production, of Bacterial Cellulose

Preparation of bacterial cellulose using tea and a tablet of Vinegar's Mother Pellicles. Requirements for the preparation of bacterial cellulose (Vinegar's Mother Pellicles). The tea specimen was activated every two weeks. The culture was prepared by adding 70 g/L of commercial sugar to tap water and after boiling, 5 g/L of dry black tea was added. The tea leaves were soaked for 15 minutes and then removed by filtration. After cooling to about 30 °C, the mother disc was added to the tea and incubated under aerobic conditions at 25 °C after 14 days only. The new disc will be formed [13].

2.2 Purification of Bacterial Cellulose

The bacterial cellulose membranes were harvested from the culture medium and subjected to multiple washing cycles (three times). The ability to purify bacterial cellulose was demonstrated by using a solution containing 0.1 M NaOH and deionized water for one hour at 80C° on a magnetic starrier. The bacterial cellulose particles washed with deionized water and left in 3% NaOCl for 24 hours, after that washed again with deionized water until reached pH 7. This process successfully removed the color and various pellicle-bound materials from bacteria, yeasts, and components of the culture medium, aiding in the analysis of bacterial cellulose properties through multiple techniques [14, 15].

2.3 Characterization of Bacterial cellulose and Bacterial nanocellulose

The characterization of cacterial cellulose and bacterial nanocellulose was carried out using transmission electron microscopy (TEM), Scanning electron microscopy (SEM), Fourior Transform Infrared Spectroscopy and (FTIR).

2.4 Specimens Collection

During the period from October 1, 2022 to December 30, 2022, a total of 150 specimens were collected from patients with wounds and burn wounds. These patients were of different age groups. It included 75 specimens of burn infections and 75 specimens of wound infections from the hospitals of the Medical City in Baghdad (Baghdad Teaching Hospital, Al-Kadhimiya Teaching Hospital, Specialized Burns Center/ Al-Yarmouk Hospital, Teaching Laboratories, and Central Child Hospital). The specimens were cultivated on several agar mediums, including nutrition agar, MacConkey agar, 5% blood agar, and mannitol salt agar. They were then incubated aerobically at 37°C for 24 hours.

2.5 Identification of Bacterial Isolates

Bacterial growth was determined by analyzing the phenotypic and cultural features and routine biochemical testing [16]. The Gram stain isolates were identified using VITEK® 2 Compact Automated Systems with ID-GN and ID-GP cards following the directions provided by the manufacturer [17].

2.6 Antibiotic Sensitivity Test

Antibiotic susceptibility testing was done in two methods:

1- Kirby Bauer Disk Diffusion Method
The method of [18] was used. The diameter of inhibition surrounding the antibiotic disk was measured to determine the effectiveness of the antibiotic.
2- AST with Vitek 2 Compact System.
The method of direct identification and susceptibility testing of positive blood cultures [19] was used. Minimum inhibitory concentrations (MICs) are determined through the automated processing of antimicrobial susceptibility cards by the VITEK-2 System.

2.7 Resazurin Microplate Assay (REMA)

REMA was used to determine (the MIC) of BC and BNC; the MIC was determined by recording the color change observed, Using Micro-titer platelet method, Mueller-Hinton medium and resazurin source dye were used [20].

2.8 Detection of Biofilm Formation

Qualitative examination of the ability of bacterial isolates to form the biofilm using a Microtiter plate Using Luria bertani broth. As detailed before, biofilm development was quantified using the 96-well tissue culture plate method (TCP) [21].

2.9 Inhibition of Biofilm Formation

Bacterial isolates were cultured on 96-well microtiter plates with two sub-inhibitory doses of bacterial cellulose (BC) and bacterial nanocellulose (BNC) using the different concentration 1000 to 1.95 µg/ml using the different concentrations 1000, 500, 250, 125, 62.5, 31.25, 15.62, 7.813.9, and 1.95 µg/ml. Planktonic bacteria were removed by washing after incubation at 37°C for 24 hours. The residual adhesive bacteria (biofilms) are dyed with crystal violet dye, enabling visualization of the biofilm using absorbance microplate Smart Reader™ (Benchmark Scientific) at 590 nm; the color intensity reflects the bacterial attachment to the plate and biofilm formation [22, 23].

2.10 DPPH Radical Scavenging Assay

A 0.135 mM DPPH solution was prepared in methanol. Subsequently, 1.0 mL of this solution was combined with 10 µL of varying concentrations of cellulose (3.125-25 mg/mL) and nanosilver cellulose (18.5-150 µg/mL). Ascorbic acid was used as a standard antioxidant as a positive control at the same concentrations for cellulose. The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 min. The absorbance of the test specimens was measured spectrophotometrically at 517 nm. These specimens were worked with duplicates. The ability to scavenge DPPH radical was calculated by the following Equation [24].

$$\text{Free radical scavenging activity (\%)} = \frac{A_{control-}\ A_{sample}}{A_{control}} \times 100$$

Where A control is the absorbance of the free radical solution DPPH + methanol, and A sample is the absorbance of the free radical solution with sample/standard antioxidant.

2.11 Statistical Analysis

The data was tabulated in a datasheet of IBM SPSS version 25.0, which was utilized to do the statistical analysis. The mean and standard errors of continuous variables were reported, and significant differences were tested using the analysis of variance (ANOVA) test, followed by the least significant difference (LSD) test. The Pearson's correlation coefficient was utilized to determine the correlation between different parameters under study. Statistical significance was defined as a probability value ($p \leq 0.05$).

3. Results

3.1 Developing Bacterial Cellulose Using Mother Pellicles of Iraqi Vinegar (BCIVMP)

Bacterial cellulose was produced from Iraqi vinegar's mother of vinegar pellicles (BCIVMP) by growing them on tea at 25 °C for 14 days. As depicted in figures 1, 13 and 15, this process formed a complete cellulose layer within the growth container within 10-20 days. The appropriate circumstances were provided for the growth of the starter disc, known as the vinegar's "mother pellicles," which included sugar as the energy source and nitrogen from black tea. A second disc of bacterial cellulose, the daughter disc, has been produced by maintaining specific temperature and pH conditions. It is typically grown on the mother's disc, as shown in Figure 1. The culture medium type, concentration, and other developmental aspects mostly influence the characteristics of the daughter disc.

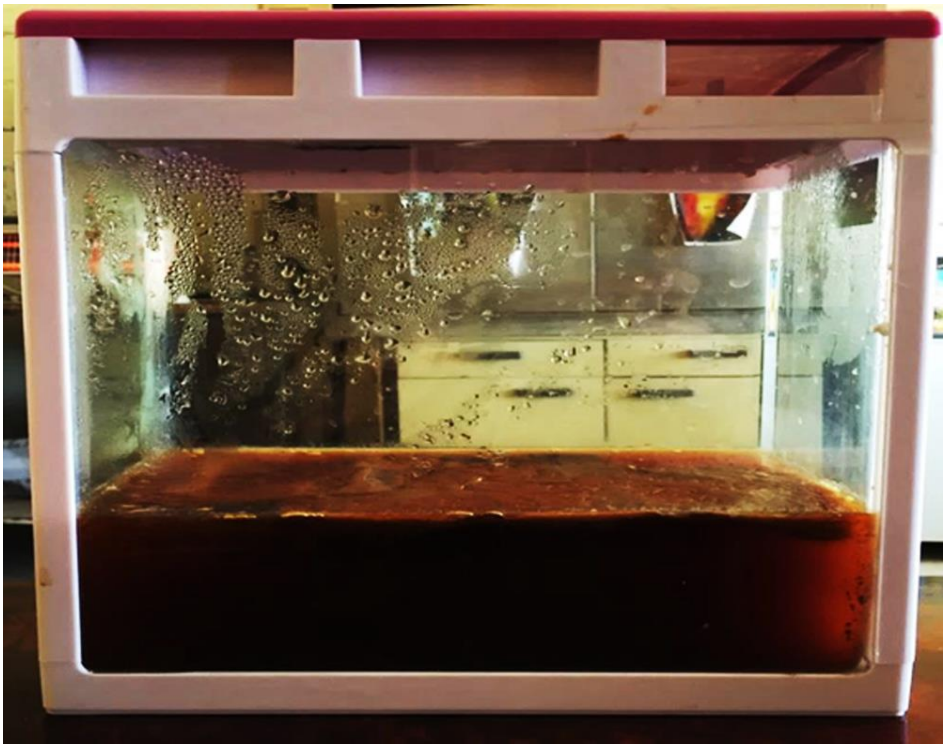

Figure 1:- Iraqi vinegar's mother pellicles grow on sweetened tea at 25 °C after 14 days.

3.2 Isolation of Bacteria

In this study, seventy bacterial isolates were collected from wounds and burn wound patients at different age groups, which included ages from 10 years to 69 years, the statistical analysis show high significance differences at $p \leq 0.001$ between each two age groups and among all groups (Table 1). The age group of 20-29 years yielded the highest number of bacterial isolates, accounting for 20 samples, which represented 29% of the total. The lowest number of isolates was collected from the age group 60-69 years, and four isolates formed a percentage of 5.71%, Table 1. The statistical analysis in Table 2 shows non-significance differences at $p > 0.05$ among all species of bacteria, where the p-value was 0.856 which is bigger than 0.05.

Table 1: The number of bacterial isolates collected from different age groups

Ages/ Years	Number of isolates	Percentage%

10-19	**10**	**14.28**
20-29	**20**	**29**
30-39	**15**	**21.42**
40-49	**12**	**17.14**
50-59	**9**	**12.85**
60-69	**4**	**5.71**
Total	**70**	**100**
$\chi 2$	**-0.576***	
Sig. (2-tailed)	**0.001***	

Table 2: The number and percentage of bacterial isolated from wounds and burn wounds patients according to age groups

Bacterial isolates	Number of isolates	Percentage
Citrobacter freundii	10	14.28 %
Escherichia coli	17	24.28 %
Klebsiella pneumonia	16	22.85%
Pseudomonas aeruginosa	15	20.83 %
Staphylococcus aureus	12	17.14 %
Total	70	100%
$\chi 2$	-0.22	
Sig. (2-tailed)	0.856[No]	

According to the information provided, the current study identified that the 70 bacterial isolates collected from wound and burn wound patients belonged to five different species, where ten isolates from *Citrobacter freundii*, seventeen isolates from *Escherichia coli*, sixteen isolates from *Klebsiella pneumoniae*, fifteen isolates from *Pseudomonas aeruginosa* and twelve isolates from *Staphylococcus aureus* were collected.

3.3 Antibiotic Susceptibility Test

The antibiotic susceptibility testing by disk diffusion method showed that most bacterial isolates of all types were resistant to the antibiotics used (Figure 2). The *Citrobacter freundii* isolates resisted the antibiotics meropenem, colistin, gentamicin, levofloxacin, imipenem, and ciprofloxacin. It is sensitive to the antibiotics amikacin and ceftazidime. As for *the Escherichia coli isolates*, they were resistant to the antibiotic meropenem, sensitive to the antibiotic amikacin, and it is moderately resistant to the rest of the types of antibiotics used. *Klebsiella pneumoniae* isolates were resistant to meropenem and moderately resistant to other antibiotics. The Pseudomonas aeruginosa isolates showed resistance to the antibiotics meropenem, colistin, and levofloxacin. They displayed moderate resistance to other antibiotics tested. Finally, *Staphylococcus aureus* isolates resisted the antibiotics meropenem, gentamycin, imipenem, and amikacin. It is moderately resistant to other antibiotics used in Table 3, and the statistical analysis illustrated significant differences at $p \leq 0.05$ between each of bacteria and antibiotics.

Table 3: Antimicrobial sensibility test by disc diffusion method.

	Number and percentage of resistance bacterial isolates

Antimicrobial agents	*Citrobacter freundii*		*Escherichia coli*		*Klebsiella pneumoniae*		*Pseudomonas aeruginosa*		*Staphylococcus aureus*	
	No.	%	No.	%	No.	%	No.	%	No.	%
MEM	10	100	17	100	16	100	15	100	12	100
CT	10	100	8	47.05	10	62.5	15	100	6	50
CN	10	100	12	70.58	13	81.25	7	46.66	12	100
LEV	10	100	1	5.88	13	81.25	15	100	3	25
IPM	10	100	7	41.17	13	81.25	8	53.33	12	100
CIP	10	100	1	5.88	7	43.75	8	53.33	3	25
AK	0	0	0	0	4	25	7	46.66	12	100
CAZ	0	0	15	88.23	10	62.5	7	46.66	9	75
$\chi 2$						**47.863**				
Sig. (2-sides)						**0.003***				

IPM: Imipenem, AK: Amikacin, LEV: Levofloxacin, CT: Colistin, MEM: Meropenem, CN: Gentamicin, CIP: Ciprofloxacin, CAZ: Ceftazidime.

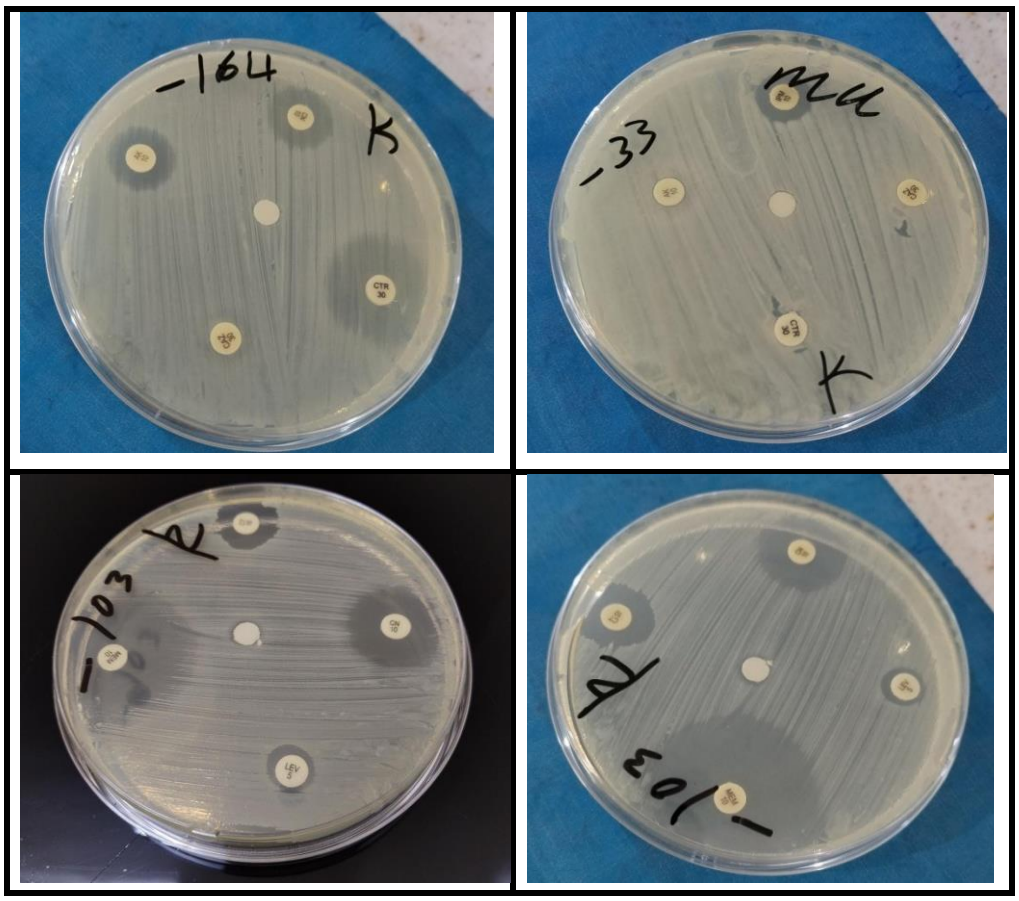

Figure 2: Antimicrobial sensibility test by disc diffusion method

The results of the antimicrobial sensibility test of Gram-negative bacteria by Vitek 2 compact system method showed that the *Citrobacter freundii* isolates were resistant to the antibiotics Ampicillin, Piperacillin/Tazobactam, Ceftriaxone, Cefoxitin, Cefepime, Imipenem, Cefazolin and Ceftazidime. It is sensitive to the antibiotics Amikacin, Levofloxacin, Gentamicin, Ciprofloxacin, Ticarcillin/Clavulanic Acid, Nitrofurantoin and Trimethoprim/Sulfamethoxazole. As for the *Escherichia coli* isolates were resistant to the

antibiotic Ampicillin and moderately resistant to the rest of the antibiotics used. *Klebsiella pneumoniae* isolates were resistant to the antibiotic Ampicillin and sensitive to the antibiotic Ticarcillin/Clavulanic Acid. It is moderately resistant to the rest of the antibiotics used. At the same time, *Pseudomonas aeruginosa* isolates showed resistance to the antibiotics Ampicillin, Piperacillin/Tazobactam, Ceftazidime, and Trimethoprim/Sulfamethoxazole. The resistance to the rest of the antibiotics used is moderate, (Table 4) and the statistical analysis shows high significance differences at $p \leq 0.001$ between each of the bacteria and antibiotics

Table 4: Antimicrobial sensibility test of Gram-negative bacteria by Vitek 2 compact system.

Antimicrobial agents	Number and percentage of resistance bacterial isolates							
	Citrobacter freundii		*Escherichia coli*		*Klebsiella pneumoniae*		*Pseudomonas aeruginosa*	
	No.	%	No.	%	No.	%	No.	%
AMP	10	100	17	100	16	100	15	100
TZP	10	100	8	47.05	10	62.5	15	100
CRO	10	100	12	70.58	13	81.25	7	46.66
FOX	10	100	1	5.88	13	81.25	15	100
FEP	10	100	7	41.17	13	81.25	8	53.33
IPM	10	100	1	5.88	7	43.75	8	53.33
AK	0	0	0	0	4	25	7	6.66
LEV	0	0	15	23	10	62.5	7	46.66
CZ	10	100	15	88.23	16	100	7	46.66
CAZ	10	100	8	47.05	10	62.5	15	100
CN	0	0	9	52.94	7	43.75	8	53.33
CIP	0	0	4	23.52	12	75	7	46.66
TGC	0	0	4	23.52	0	0	7	46.66
F	0	0	9	52.94	13	81.25	8	53.33
SXT	0	0	4	23.52	10	62.5	15	100
$\chi 2$	**108.209**							
Sig. (2-sides)	**0.000***							

AMP: Ampicillin, TZP: Piperacillin/Tazobactam, CRO: Ceftriaxone, FOX: Cefoxitin, FEP: Cefepime, IPM: Imipenem, AK: Amikacin, LEV: Levofloxacin, TGC: Ticarcillin/Clavulanic Acid, CAZ: Ceftazidime, CN: Gentamicin, CIP: Ciprofloxacin, CZ: Cefazolin, F: Nitrofurantoin, SXT: Trimethoprim/Sulfamethoxazole.

The results of the antimicrobial sensibility test of Gram-positive bacteria by Vitek 2 compact system method showed that the *Staphylococcus aureus* isolates were resistant to Piperacillin, Amoxicillin, Levofloxacin, Tetracycline, and Nitrofurantoin. It is sensitive to the antibiotics Tobramycin, Linezolid, Vancomycin, Ticarcillin/Clavulanic Acid, Rifampicin, and Gentamicin. It is moderately resistant to Fusidic Acid and Clindamycin and the statistical analysis shows Significant differences at $p \leq 0.05$ between all antibiotics Table 5.

Table 5: Antimicrobial sensibility test of Gram-positive bacteria by Vitek 2 compact system

Antimicrobial agents	*Staphylococcus aureus*

	No.	%
PIP	**12**	**100**
AMX	**12**	**100**
OX	**12**	**100**
TM	**0**	**0**
MXF	**0**	**0**
LNZ	**0**	**0**
VA	**0**	**0**
TGC	**0**	**0**
FA	**5**	**41.66**
RA	**0**	**0**
CN	**0**	**0**
LEV	**12**	**100**
CM	**10**	**83.33**
TE	**12**	**100**
F	**12**	**100**
$\chi 2$	**-.291**	
Sig. (2-tailed)	**0.008***	

PIP: Piperacillin, AMX: Amoxicillin, Ox: Oxacillin, TM: Tobramycin, MXF: Moxifloxacin, LNZ: Linezolid, VA: Vancomycin, TGC: Ticarcillin/Clavulanic Acid, FA: Fusidic Acid, RA: Rifampicin, CN: Gentamicin, LEV: Levofloxacin, CM: Clindamycin, TE: Tetracycline, F: Nitrofurantoin

3.4 Antimicrobial Activity of Bacterial Cellulose and Bacterial Nanocellulose by Resazurin

The antimicrobial activity of bacterial cellulose on different bacterial isolates by Resazurin-based 96-well plate microdilution method showed that the minimum inhibitory concentration (MIC) of bacterial cellulose for *Citrobacter freundii* was between (1000-15.62µg/ml), for *Escherichia coli* was between (500-15.62µg/ml), for *Klebsiella pneumonia* was between (500-15.62µg/ml), for *Pseudomonas aeruginosa* was between (250-15.62µg/ml) and for *Staphylococcus aureus* was between (500-15.62µg/ml, Figure 3), while Table 6 shows the mean and standard error of bacterial Cellulose and nano-cellulose with the p-value, where there are Significance differences at $p \leq 0.05$ between bacterial cellulose and nano-cellulose in MIC.

Table 6: The MIC values of antimicrobial activity of bacterial cellulose on different bacterial isolates

	Cellulose Mean±SE	Nano-cellulose Mean±SE	P value between groups
Citrobacter freundii	50.6240±11.53230	22.6240±5.12769	0.010*
Escherichia coli	33.2706±4.24527	28.5647±4.30838	0.570No
Klebsiella pneumonia	41.6105±6.31418	27.1636±4.55470	0.091No
Pseudomonas aeruginosa	47.9740±9.28518	31.1073±5.49248	0.050*
Staphylococcus aureus	37.0825±5.98026	29.0617±6.23487	0.416No
P value	0.050*		

The antimicrobial activity of bacterial nanocellulose on different bacterial isolates by Resazurin-based 96-well plate microdilution method showed that the minimum inhibitory concentration (MIC) of Bacterial Nanocellulose for *Citrobacter freundii* was between (1000-15.62µg/ml), for *Escherichia coli* was between (1000-15.62µg/ml), for *Klebsiella pneumonia* was between (1000-15.62µg/ml), for *Pseudomonas aeruginosa* was between (1000-15.62µg/ml) and for *Staphylococcus aureus* was between (1000-31.25µg/ml) Figure 4.

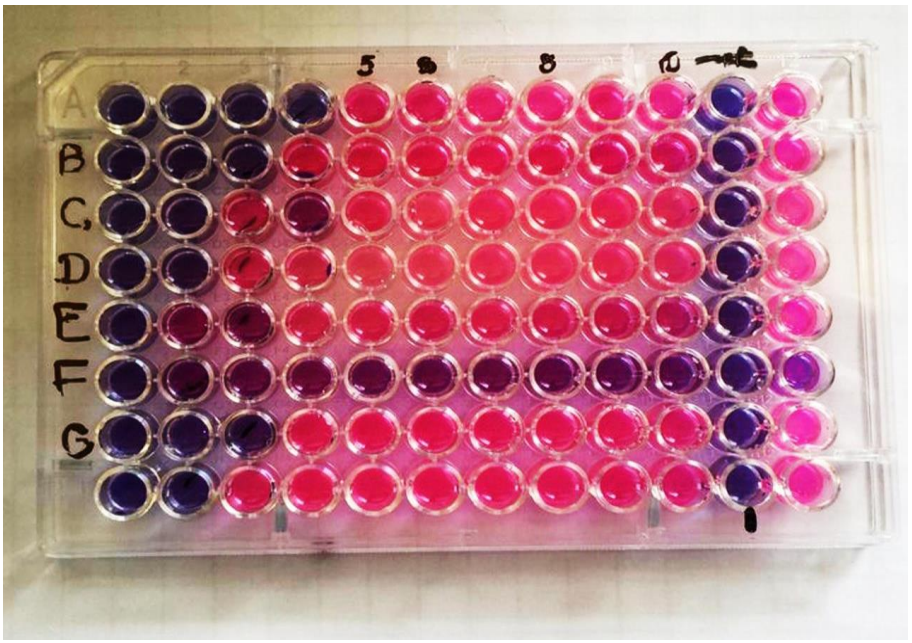

Figure 3: The antimicrobial activity of bacterial nanocellulose on different bacterial isolates by Resazurin-based 96-well plate microdilution method.

3.5 Biofilm Formation Detection

The microtiter plate method revealed that 85.71% (60 out of 70) of the bacterial isolates were biofilm producers, while the remaining 14.29% (10 isolates) did not produce biofilm, as illustrated in Figure 4.

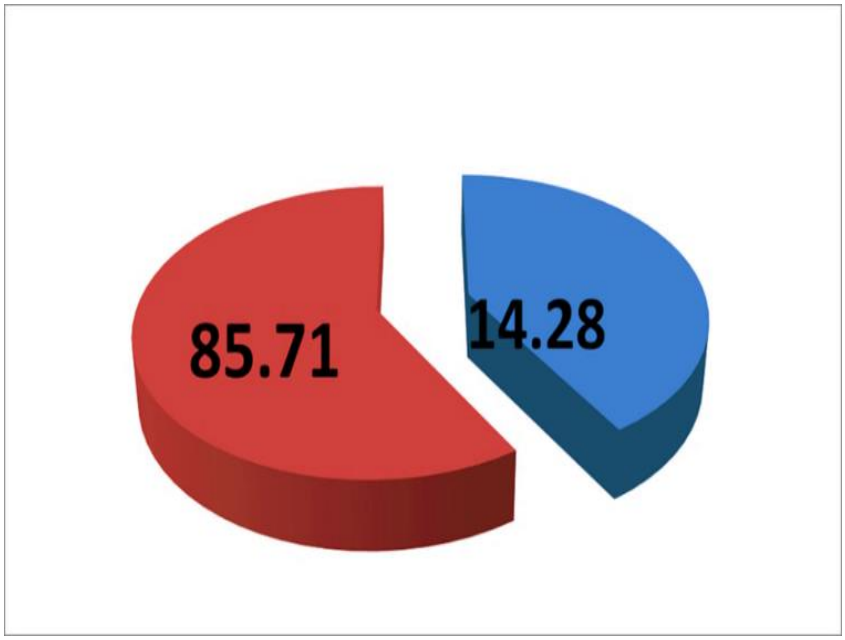

Figure 4: The biofilm formation by bacterial isolates, blue not biofilm-forming isolates, read strong-biofilm-forming isolates.

The biofilm-forming isolates were divided into 25 strong-biofilm-forming isolates, with a percentage of 41.66%, twenty moderate-biofilm-forming isolates, with a percentage of 33.33%, and fifteen isolates of weak biofilm-forming, with a percentage of 25%, (Figures 5).

Table 7 illustrates the mean and standard error for the formation of biofilm. There are Non-significance differences at p>0.05 between bacterial cellulose and nano-cellulose in biofilm formation.

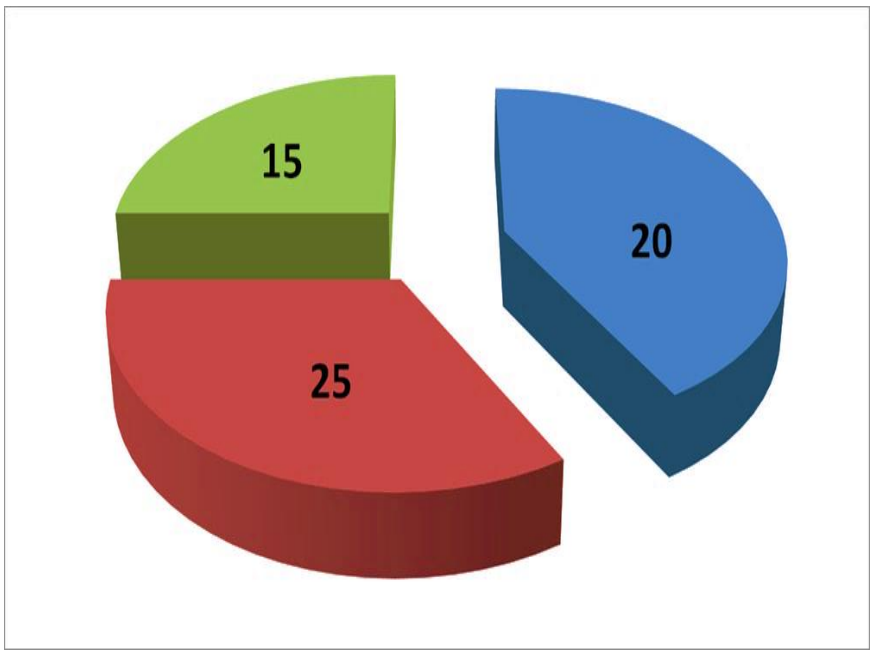

Figure 5: The biofilm-forming isolates included 25 strong-biofilm-forming isolates, 20 moderate-biofilm-forming isolates, and 15 isolates of weak biofilm-forming

Table7: The biofilm formation of different bacterial isolates by microtiter plate method

	Mean±SE	Biofilm formation
Citrobacter freundii	0.1764±0.03142	Moderate
Escherichia coli	0.1458±0.02119	Moderate
Klebsiella pneumonia	0.1558±0.02327	Moderate
Pseudomonas aeruginosa	0.1365±0.02493	Moderate
Staphylococcus aureus	0.2003±0.02535	Strong
P value	0.416^{No}	

4.6 The Effect of Bacterial cellulose and Bacterial nanocellulose on The Biofilm formation

The results of the effect of bacterial cellulose on biofilm formation showed that SUB-MIC 1 of bacterial cellulose prevented biofilm formation in seventeen isolates, and the percentage of inhibition was 78%. Whereas the use of SUB-MIC 2 of bacterial cellulose prevents the biofilm formation in ten isolates, the percentage of inhibition was 42% Figures 6 and 7.

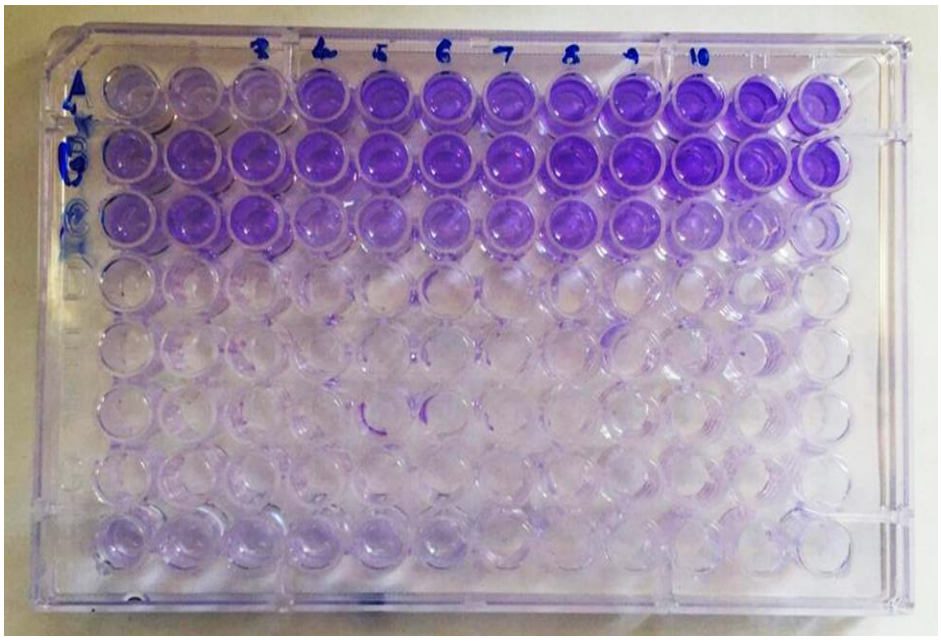

Figure 6: The effect of bacterial cellulose on biofilm formation by microtiter plate method

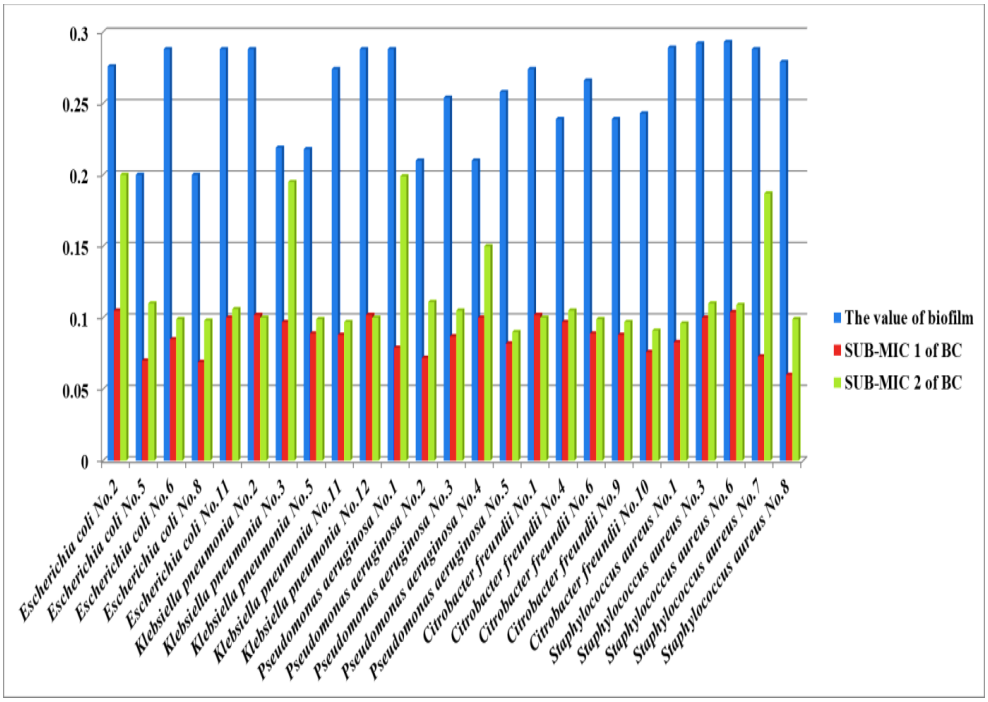

Figure 7: The effect of bacterial cellulose on biofilm formation

The results of the effect of bacterial nanocellulose on biofilm formation showed that using SUB-MIC 1 of bacterial nanocellulose prevents the biofilm formation in twenty-five isolates; the percentage of inhibition was 100%. The use of SUB-MIC 2 of bacterial cellulose prevented biofilm formation in 23 isolates, representing an 80% inhibition rate (as shown in Figures 8 and 9). Table 8 shows the mean and standard error for SUB-MIC 1, where there are significant differences at $p>0.05$ between bacterial cellulose and nano-cellulose in SUB-MIC 1. While table 9 shows non-significant differences at $p>0.05$ between bacterial cellulose and nano-cellulose in SUB-MIC 2.

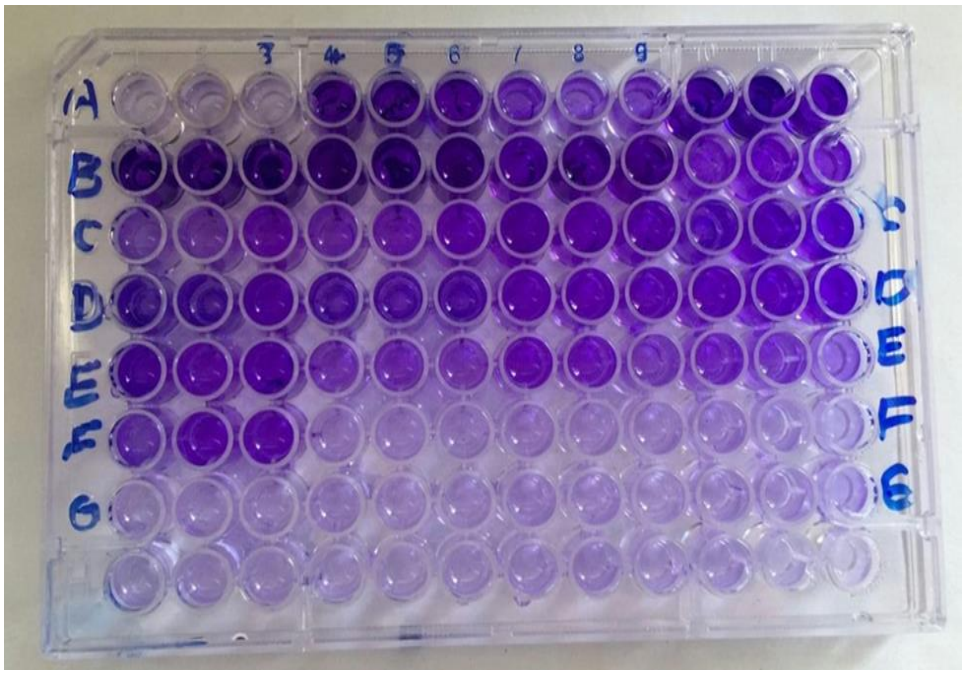

Figure 8: The effect of bacterial nanocellulose on biofilm formation.

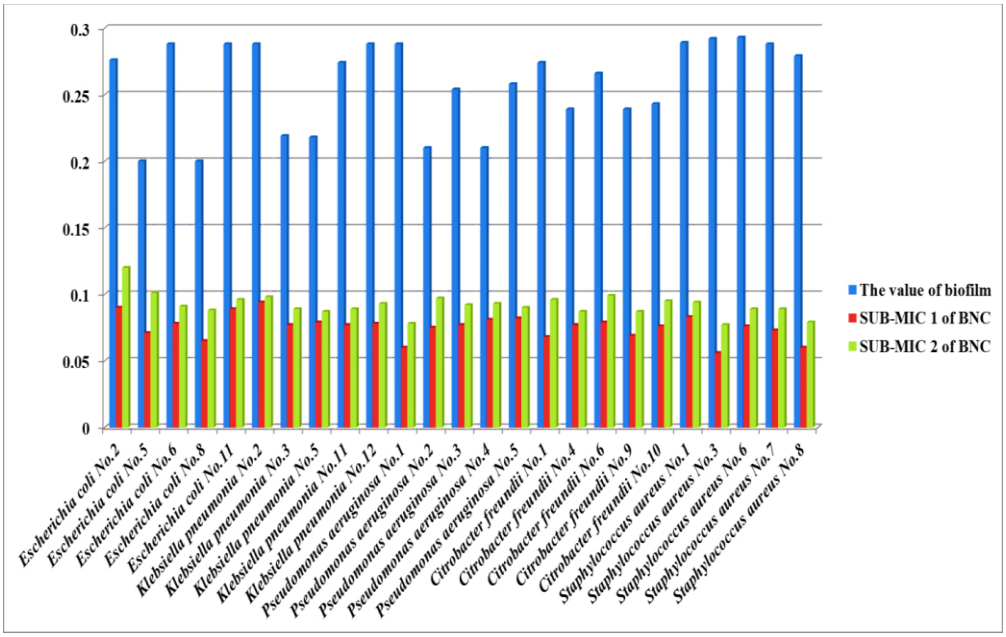

Figure 9: The effect of bacterial nanocellulose on biofilm formation

Table 8: The effect of bacterial cellulose and nano-cellulose on biofilm formation in SUB-MIC 1

	Cellulose **Mean±SE**	Nano-cellulose **Mean±SE**	**P value between groups**
Escherichia coli	0.0858±0.00743	0.0786±0.00491	0.319^{No}
Klebsiella pneumonia	0.0956±0.00304	0.0810±0.00327	0.047*
Pseudomonas aeruginosa	0.0840±0.00468	0.0750±0.00396	0.214^{No}
Citrobacter freundii	0.0904±0.00443	0.0738±0.00222	0.025*
Staphylococcus aureus	0.0840±0.00823	0.0696±0.00505	0.050*
P value	0.025*		

Table 9: The effect of bacterial cellulose and nano-cellulose on biofilm formation in SUB-MIC 2

	Cellulose **Mean±SE**	Nano-cellulose **Mean±SE**
Escherichia coli	0.1226±0.01948	0.0992±0.00565
Klebsiella pneumonia	0.1182±0.01921	0.0912±0.00196
Pseudomonas aeruginosa	0.1310±0.01967	0.0900±0.00321
Citrobacter freundii	0.0984±0.00227	0.0928±0.00246
Staphylococcus aureus	0.1202±0.01692	0.0856±0.00325
P value	0.099^{No}	

4.7 The Antioxidant Activity of Bacterial Cellulose, Bacterial Nanocellulose, and Ascorbic Acid

The results of the antioxidant activity of bacterial cellulose, bacterial nanocellulose, and ascorbic acid at different concentration by PDDH radical scavenging activity showed that % PDDH radical scavenging activity increased with an increase in the concentration of BC, BNC, and AA. The % PDDH radical scavenging activity for bacterial cellulose was 10.316 when the concentration was 1.95µg/ml. The percentage inhibition becomes 75.122 when the concentration is 1000µg/ml. Based on the information provided, the % DPPH) radical scavenging activity of bacterial nanocellulose was 14.806% when the concentration of the sample was 1.95 µg/ml. The percentage inhibition becomes 98.837 when the concentration is 1000µg/ml. The % PDDH radical scavenging activity for ascorbic acid was 16.213 when the concentration was 1.95µg/ml. The percentage inhibition becomes 98.940 when the concentration is 1000µg/ml and Figure 10. The statistical analysis shows non-significance differences at p>0.05 among bacterial cellulose, bacterial nanocellulose, and ascorbic acid OD (Table 10).

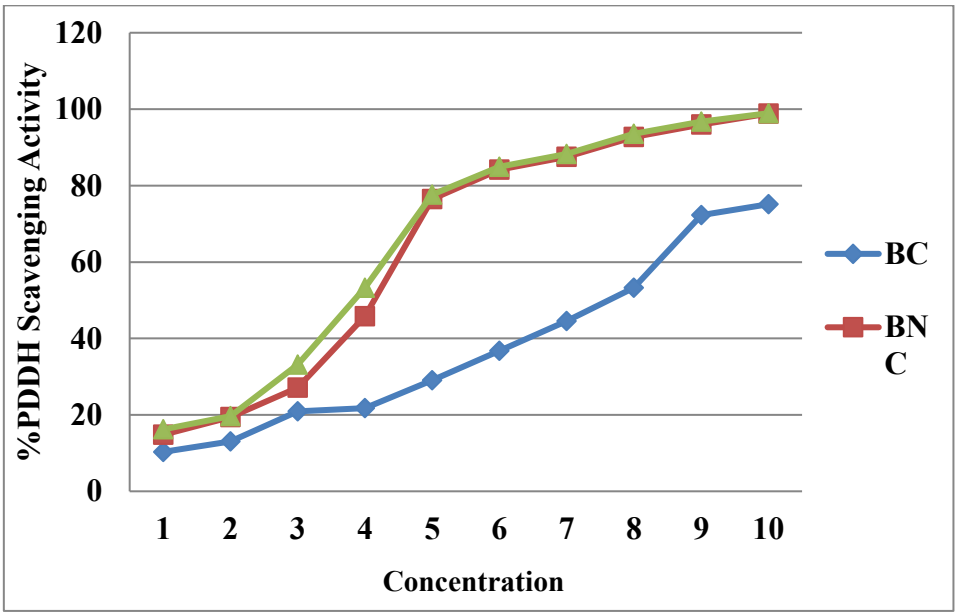

Figure 10: Percentage inhibition of bacterial cellulose, bacterial nanocellulose, and ascorbic acid.

Table 10: The absorption and percentage inhibition of bacterial cellulose, bacterial nanocellulose, and ascorbic acid at different concentrations, with the absorption of control=1.265

Concentration µg/ml	Bacterial cellulose OD	Percentage inhibition	Bacterial nanocellulose OD	Percentage inhibition	Ascorbic acid OD	Percentage inhibition
1000	0.3147	75.122	0.0147	98.837	0.0134	98.940
500	0.3512	72.237	0.0512	95.952	0.0412	96.743
250	0.5917	53.225	0.0917	92.750	0.0817	93.541
125	0.7011	44.577	0.1579	87.517	0.1479	88.308
62.5	0.7998	36.774	0.1998	84.205	0.1911	84.893
31.25	0.8975	29.051	0.2975	76.482	0.2824	77.675
15.62	0.9899	21.747	0.6854	45.818	0.5911	53.272
7.81	1.0000	20.948	0.9222	27.098	0.8456	33.154
3.9	1.1000	13.043	1.0199	19.375	1.0167	19.628
1.95	1.1345	10.316	1.0777	14.806	1.0599	16.213

4. Discussion

The present study identified seventy bacterial isolates from wound and burn patients, encompassing a diverse age range of 10 to 69 years. The highest number of bacterial isolates was 20, with a rate of 21.42%, and they were collected from the age group 30-39 years. The lowest number of isolates was collected from the age group 60-69 years, and it was four, forming a percentage of 5.71%. These results agree with several studies [25, 26] that found the same results. Seventy bacterial isolates belong to five species, where ten isolates from *Citrobacter freundii*, seventeen isolates from *Escherichia coli*, sixteen isolates from *Klebsiella pneumoniae*, fifteen isolates from *Pseudomonas aeruginosa* and twelve isolates from *Staphylococcus aureus* were collected from burn wounds and wounds of patients attending Baghdad hospitals in Karkh and Rusafa. The results found in this study are aligned with those reported by [27], in which the same bacterial types were isolated from burn wounds and wounds of patients visiting hospitals in Baghdad. The antibiotic susceptibility testing by disk diffusion method showed that most bacterial isolates of all types were resistant to the antibiotics used; the *Citrobacter freundii* isolates were resistant to the antibiotics meropenem, colistin, gentamicin, levofloxacin, imipenem, and ciprofloxacin. It is sensitive to the antibiotics amikacin and ceftazidime. As for the *Escherichia coli* isolates, they were resistant to the antibiotic meropenem, sensitive to the antibiotic amikacin, and it is moderately resistant to the rest of the types of antibiotics used. *Klebsiella pneumoniae* isolates were resistant to meropenem and moderately resistant to other antibiotics. *Pseudomonas aeruginosa* isolates resisted the antibiotics meropenem, colistin, and levofloxacin. It is moderately resistant to other antibiotics used. Finally, *Staphylococcus aureus* isolates resisted the antibiotics meropenem, gentamycin, imipenem, and amikacin. These results are consistent with the findings of [28-30]. The same observations were observed, as they found that bacterial isolates of all kinds increased their resistance to antibiotics. The increase in antibiotic resistance is due to the indiscriminate use of antibiotics and failure to complete the course, which provides greater opportunities for bacteria to overcome antibiotics through genetic mutations and adaptation [31].

The results of the antimicrobial sensibility test of Gram-negative bacteria by Vitek 2 compact system method showed that the *Citrobacter freundii* isolates were resistant to the antibiotics Ampicillin, Piperacillin/Tazobactam, Ceftriaxone, Cefoxitin, Cefepime, Imipenem,

Cefazolin and Ceftazidime. It is sensitive to the antibiotics Amikacin, Levofloxacin, Gentamicin, Ciprofloxacin, Ticarcillin/Clavulanic Acid, Nitrofurantoin, and Trimethoprim/Sulfamethoxazole. As for the *Escherichia coli* isolates were resistant to the antibiotic Ampicillin and moderately resistant to the rest of the antibiotics used. *Klebsiella pneumoniae* isolates were resistant to the antibiotic Ampicillin and sensitive to the antibiotic Ticarcillin/Clavulanic Acid. It is moderately resistant to the rest of the antibiotics used. At the same time, *Pseudomonas aeruginosa* isolates showed resistance to the antibiotics Ampicillin, Piperacillin/Tazobactam, Ceftazidime, and Trimethoprim/Sulfamethoxazole. The resistance to the rest of the antibiotics used is moderate. The results of the antimicrobial sensibility test of Gram-positive bacteria by Vitek 2 compact system method showed that the *Staphylococcus aureus* isolates were resistant to Piperacillin, Amoxicillin, Levofloxacin, Tetracycline, and Nitrofurantoin. It is sensitive to the antibiotics Tobramycin, Linezolid, Vancomycin, Ticarcillin/Clavulanic Acid, Rifampicin, and Gentamicin. It is moderately resistant to Fusidic Acid and Clindamycin. These results are consistent with many studies [32-34]. These reported increased antibiotic resistance for bacterial isolates from wounds and burns in recent years.

By employing these two complementary methods, the researchers were able to assess the antimicrobial sensitivity of the bacterial isolates using both a traditional disk diffusion technique and an automated, high-throughput system. The sensitivity test with the AST with Vitek 2 compact system gives accurate results compared to the Kirby Bauer Disk Diffusion Method. These results are consistent with many studies [35-37]. These mentioned some drawbacks of the Kirby Bauer Disk Diffusion Method, such as the bacteria not being distributed evenly on the plate and the possibility of contamination of the Petri dishes. At the same time, the AST with Vitek 2 compact system is almost free of drawbacks, in addition to its ease, accuracy, and speed. The antimicrobial activity of bacterial cellulose and bacterial nanocellulose on different bacterial isolates by Resazurin-based 96-well plate microdilution method showed that the minimum inhibitory concentration (MIC) of Bacterial Cellulose and bacterial nanocellulose for *Citrobacter freundii* was between (1000-15.62µg/ml), for *Escherichia coli* was between (500-15.62µg/ml) and (1000-15.62µg/ml), for *Klebsiella pneumonia* was between (500-15.62µg/ml) and (1000-15.62µg/ml), for *Pseudomonas aeruginosa* was between (250-15.62µg/ml) and (1000-15.62µg/ml), and for *Staphylococcus aureus* was between (500-15.62µg/ml) and (1000-31.25µg/ml). These results agree with many studies [38-41]. These have proven that bacterial cellulose and nanocellulose have antibacterial activity for Gram-positive and Gram-negative bacteria. The resazurin microplate assay (REMA) is simple, inexpensive, and rapid and might be used with other drugs. The REMA method allows accurate MIC measurements while overcoming critical issues related to color and solubility, which may interfere with growth measurements for many biosurfactant extracts.

This study showed that sixty of the seventy bacterial isolates produced biofilm, which was detected by the microtiter plate method, making up a percentage of 85.71%, and ten isolates were not biofilm-producing, making up a percentage of 14.28%. The biofilm-forming isolates were divided into 25 strong-biofilm-forming isolates, with a percentage of 41.66%, twenty moderate-biofilm-forming isolates, with a percentage of 33.33%, and fifteen isolates of weak biofilm-forming, with a percentage of 25%. These results are consistent with many studies [42-45]. These have proven that bacteria isolated from burns and wounds tend to form a biofilm that helps the bacteria adhere to and resist therapeutic materials such as antibiotics and antiseptics. The effect of the BC and BNC on the biofilm formation using SUB-MIC 1 shows that BC and BNC prevented the biofilm formation in 17 and 25 isolates; the percentage of inhibition was 78% and 100%, respectively. Meanwhile, SUB-MIC 2 of BC and BNC prevented the biofilm formation in 10 and 23 isolates, respectively. The percentage of inhibition was 42% and 80%. These results agree with several studies [46, 47]. These

demonstrated that bacterial cellulose and nanocellulose can influence bacterial virulence factors such as biofilm formation. The results of the antioxidant activity of bacterial cellulose, bacterial nanocellulose, and ascorbic acid at different concentration by PDDH radical scavenging activity showed that % PDDH radical scavenging activity increased with an increase in the concentration of BC, BNC, and AA. The % PDDH radical scavenging activity for bacterial cellulose was 10.316 when the concentration was 1.95µg/ml. The percentage inhibition becomes 75.122 when the concentration is 1000µg/ml. The % PDDH radical scavenging activity for bacterial nanocellulose was 14.806 when the concentration was 1.95µg/ml. The percentage inhibition becomes 98.837 when the concentration is 1000µg/ml. The % PDDH radical scavenging activity for ascorbic acid was 16.213 when the concentration was 1.95µg/ml. The percentage inhibition becomes 98.940 when the concentration is 1000µg/ml. These results are consistent with many studies [48-52], which proved that bacterial cellulose has an effective free radical scavenging activity. Still, it is less effective than nanocellulose free radical scavenging, which approached the results of free radical scavenging by ascorbic acid, the experiment's standard material, to measure the materials' antioxidant activity.

Conclusions

The current study demonstrates that bacterial cellulose and bacterial nanocellulose exhibit antimicrobial, antivirulence, and antioxidant properties, enabling their application across a broad spectrum of medical, industrial, and environmental fields. This study recommends the possibility of studying cellulose and bacterial nano-cellulose in a synergistic manner with antibiotics. In addition to using nanoparticles of metals other than silver and comparing them with silver nanoparticles loaded on bacterial cellulose.

Acknowledgment

We thank the Ministry of Science and Technology staff, especially Dr. Labib and his group, for their assistance in conducting the experiments and interpreting the results

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