

Biological and inhibitory activity of carbon nanotubes against the biofilm produced by Enterobacteriaceae

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Abstract:

Multiple drug resistance prompted researchers to investigate novel components that might effectively restrict the development of microorganisms, and carbon nanotubes were employed to suppress the biofilm in this study. Bacteria in a biofilm develop differently than bacteria in a planktonic environment. The antimicrobial test was performed using Perez and others' approach, the carbon nanotube test (0.05 percent, 0.02 percent, 0.01 percent). The concentration was tested. In addition, Enterobacteriaceae reveals that the average inhibitory zone is 54 mm. In addition, pathogenic isolates could not form biofilms when exposed to carbon nanotubes. *Klebsiella pneumonia* had a maximum value of 0.426 nm and then dropped to 0.171 nm. The inhibitory impact of carbon- nanotube and the suppression of biofilm formation by pathogenic bacteria were discovered in this study.

Keywords: Enterobacteriaceae, carbon nanotubes, biofilm formation.

1. Introduction

Multidrug resistance MDR has lately grown among gram-negative pathogens such as extended-spectrum beta-lactamase-producing (ESBL-producing) Enterobacteriaceae strains (Poole,2004). The Enterobacteriaceae family is found naturally in people and animals' digestive tracts; it is a diverse group of Gram-negative bacteria. Urinary tract infections (UTIs) are the most prevalent hospital-acquired infections caused by Enterobacteriaceae, but lower respiratory tract and bloodstream infections are fatal. (2010); Magiorakos et al., 2017). Enterobacteriaceae have virulence factors such as diverse adhesions, serum resistance, and biofilm formation; these factors, particularly the ability to form biofilms in the human gut, contribute to gut colonization and significantly impact the function and interactions of the gut microbiome with the host. (Sicard et al., 2017; Rossi et al., 2017; Sicard et al., 2017).

A biofilm is an organized population of bacteria that attaches to an inert or live surface and is surrounded by a self-producing matrix. The stimulation of biofilm formation and the survival of bacterial populations in hospitals and patients have increased the likelihood of infectious diseases (Greene,2016). Previous research found a link between antimicrobial resistance genes and biofilm in *E. coli* and *Klebsiella* strains isolated from individuals with various illnesses (Alcantar-Curiel et al.,2018). Several studies have shown that antibiotics have a role in biofilm management and that low dosages of particular antibiotics may induce biofilm

development. (Kaplan, 2011) The issue of multidrug resistance has prompted researchers to investigate novel components that might effectively suppress the development of germs; it has been known that carbon-based nanoparticles have strong antimicrobial properties. The ability of nanoparticles to interact with bacteria improves when reducing their size. Carbon nanoparticles' size and surface area are critical elements determining their antibacterial action. (Kang,2008).

2. Material and methods:

2.1.Preparation of concentration of nanotubes

Prepare three different concentrations of the (Stock solution) by dissolving 25 mg of carbon nanotube in 100 ml of distilled water (0.05 percent, 0.02 percent, 0.01 percent).

2.2.Preparation of the Bacterial Suspension

25 Urine samples were obtained from patients with urinary tract infections at Al-Sadr Teaching Hospital in Najaf and tested on Chrom agar medium. Each bacterial suspension's turbidity was adjusted to meet the standard of 0.5 McFarland (1.5×10^8 CFU / ml). Turbidity was assessed using a spectrophotometer at 625 nm in turbid suspension using the Bauer-Kirby Method (1966).

2.3.Detection of antimicrobial activity

Using Chrom agar medium, E.coli, Klebsiella pneumonia, Acinetobacter baumannii, and Proteus mirabilis were isolated and identified. Spread 0.1ml of the culture on Muller Hinton Agar with a sterile swab and dry at room temperature for (10-15) minutes; the agar well diffusion method (Perez et al. 1990) was used; four wells with a diameter of 10 mm were made on the surface of the culture media, 100 μ l of different concentrations of carbon nanotube were added to each well, and antibiotic tablets (5 micrograms) were added for comparison with the carbon nanotube concentrations.

2.4.Inhibition of biofilm formation

200 μ l of BHI medium were transferred to (MTP 96-wells), 100 μ l of nanoparticles, and 20 μ l of suspension to each well that contained culture media prepared with the use of a control well containing only (BHI) broth; the plates were incubated at 37 ° C for 18-24 hours. The contents of the wells were removed and washed 3 times with buffer solution phosphates, and ethanol-adherent cells were stabilized at 95% concentration and left for 10 minutes. The wells were dyed with Crystal violet at a concentration of 1%, and 100 μ l of dye was added to each hole for 15 minutes. Then the well was washed with sterile distilled water three times to remove the non-sticking dye. Thus, the ability of bacteria to adhere quantitatively can be estimated by observing the amount of dye attached to the wells, and to qualitatively estimate the ability of bacteria to produce the mucous material, the adhesive dye was removed by adding 200 μ l at a concentration of 100% of methanol to each hole. Absorbance was measured at the wavelength of 630 nm with an ELIZA instrument.

2.5.Statistical analysis:

The data was obtained and exported to a Microsoft Excel spreadsheet, used to conduct descriptive statistics. SAS version 9.1 was used to analyze and handle the data. Additionally, a two-way ANOVA was used to assess any interaction between extract concentration and pathogenic microorganisms. A P value of 0.05 is considered significant (Tukey test).

3. RESULT AND DISCUSSION

3.1.

This study showed that the biofilm significantly decreased according to the bacterial isolates after treatment with the carbon nanotube compared to the control treatments, as (A) *Klebsiella pneumonia* had the highest value of 0.171 nm, decreased to 0.0363 nm, As in Figure 1.

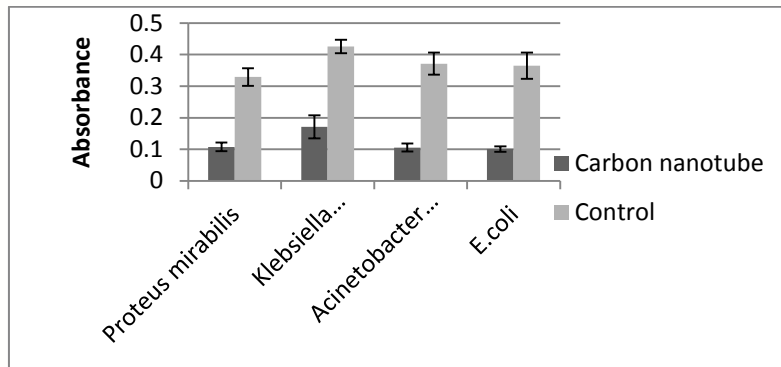


Figure 1: The (X) axis represents the pathogenic bacterial isolates, and the (Y) axis represents the readings at the wavelength of 630 nm using an ELIZA device. The ability of bacteria to adhere to wells can be quantified by observing the amount of dye adhering to the wells. Figure (2).

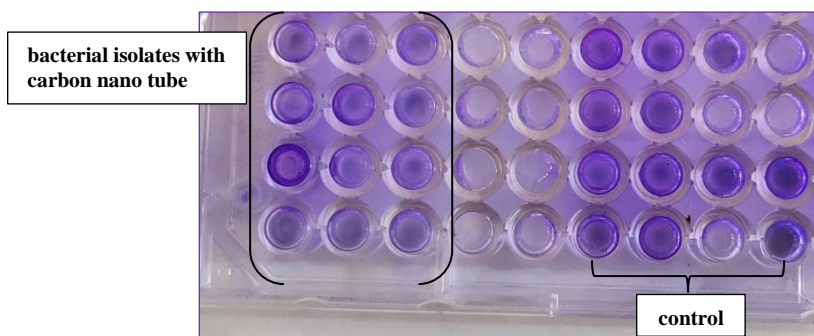


Figure (2): shows the treatment of the bacterial isolates with the carbon nanotube, where the transparent gradient of the dye is observed because it caused the biofilm breakdown.

The actual cause of infection in both the community and hospitals are Gram-negative microorganisms (Kallen and Srinivasan,2010). Biofilm formation is a critical characteristic of Enterobacteriaceae (Nielsen et al.,2018). In this study, we showed, as in Figure (1), that the concentration of biofilm production of bacterial isolates decreased significantly after treatment with the nanocomposite, as *Klebsiella pneumonia* had the highest value of (0.171) nm decreased (0.426) nm, The other one that showed a lesser decrease was *Proteus mirabilis*, *Acinetobacter baumannii* and *E.coli* (0.107, 0.105 and 0.100) respectively. As from Figure 1, the *Klebsiella pneumonia* has the highest value in biofilm production and can grow at low temperatures, low nutrient concentrations in the presence of *Klebsiella pneumonia* can rapidly use nutrients in biofilms, so it adapts well to biofilm formation in chemical

and physical environments it is found in distribution systems (Vuotto et al.,2017; Ostria-Hernandez et al.,2018; Camper et al.,1991). Carbon nanotube has a good antibacterial and anti-biofilm effect on UTIs bacteria. Due to the formation, the bacterial biofilm occurs in three stages: initial adsorption, irreversible adhesion to the surface, and colonization (Kolter and Greenberg,2006). In Figure 2, the biofilm formation of the pathogenic isolates significantly decreased after treatment with carbon nanotubes compared with control, and because of concentration of nanotubes is toxic as they lead to the release of intracellular substances, such as DNA and RNA to cells of bacteria by damaging the cell wall (Kang et al.,2008). on the other hand, the inhibition of biofilm formation from spores was most likely through the combination of reduced germination of spores and the inhibition of subsequent vegetative cell multiplication (Kang et al.,2008).

3.2. Antimicrobial assay

In this study, three concentrations (0.05%, 0.02%, and 0.01%) of carbon nanotubes were carried out against most of the tested isolates (*E. Coli*, *Klebsiella pneumonia*, *Acinetobacter baumannii*, and *Proteus mirabilis* compared with the antibiotic ciprofloxacin (5 μ g). The inhibition zone appeared at 54 mm at the concentration (0.05%) as in Figure 3, while at the concentrations (0.02%, 0.01%), the inhibitory zone against pathogenic isolates seemed to be immeasurable (very small). It also showed that the inhibition zone of carbon nanotubes was more significant than the area of antibiotic inhibition, and there was a synergy between them (Fig. 4)

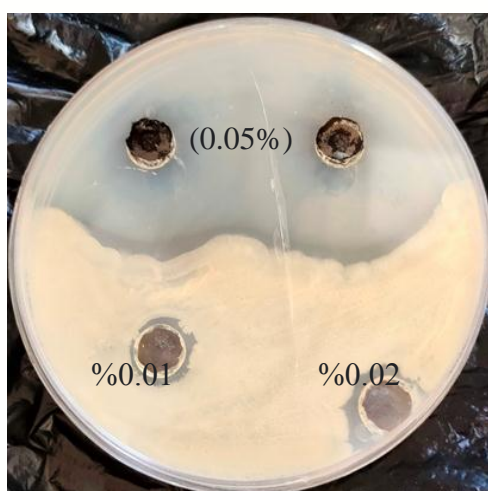


Fig. 3 Inhibition zones formed by the carbon nanotubes at the concentration (0.05%)

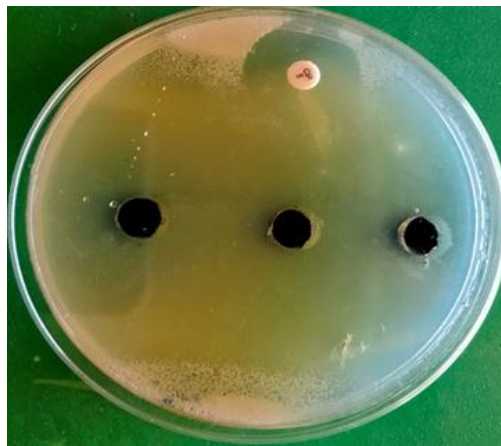


Fig. 4 Inhibition zones formed by the carbon nanotubes at concentration compared with the antibiotic (0.05%).

Studies found that CNTs have great antibacterial potential, proven in current research. Zardini et al. 2014 (Zardini et al.,2014) demonstrated antimicrobial activity against four-gram positive and four-gram negative strains. The authors also described the main antibacterial mechanisms of CNTs: Oxidation of the membrane leads to disruption of the integrity of the membrane due to strong electrostatic forces between the microbial outer surface and CNTs, direct damage to the biological particles of bacteria, and indirect DNA destruction can result from the generation of reactive oxygen species (Jackson et al.,2013; Yang et al.,2010; Lawrence et al.,2016). On the other hand, the synergistic effect of both antibiotics and carbon increases the antibacterial property as the bacteria showed resistance to antibiotics in recent years. Carbon nanotubes are suitable carriers for peptides, proteins, and genes as these macromolecules get easily degraded by enzymes present on the cell surface or inside(Nigar Anzar et al.,2020).

4. Conclusion

Carbon nanotubes can be used, which has many applications, and because of properties that make them a suitable material for biomedicine because it's more biocompatible. Carbon nanotubes work as carriers for the effective delivery of biomolecules such as antibiotics, proteins, DNA, RNA, immunocomplexes, lectins, and biosensors, which have thus shown unique advantages. Future research should investigate the most effective CNT-based devices to improve human health and interest in a disease-free life.

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6. Conflict of Interest: None

6. References

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