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RESEARCH ARTICLE

Synthesis of C-dot by hydrothermal method and evaluation of its anti-bacterial effect against antibiotic resistant *S. areus* and *K. pneumonea*

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ABSTRACT

Objective(s): Carbon dots (C-dots) are an emerging class of engineered nanomaterials with broad applications in medicine, bio-imaging, sensing, electronic devices, and catalysis. The study aimed to synthesize carbon nanoparticles with antibacterial therapeutic properties against clindamycin-resistant *Staphylococcus aureus* and ciprofloxacin-resistant *Klebsiella pneumonia strains*.

Methods: The C-dots were prepared by a hydrothermal method. Then the synthesized carbon dot were characterized by UV-visible spectroscopy, dynamic light scattering, Fourier transform infrared spectroscopy and transmission electron microscopy. The minimum inhibitory concentration of C-dots was evaluated by the micro-broth dilution method. Antibiotic susceptibility testing was performed using the disk diffusion method.

Results: The C-dots significantly reduced S. aureus and *K. pneumoniae* strains growth when compared to untreated bacteria (control; $P < 0.05$). Therefore, the minimum inhibitory concentration (MIC) of C-dots for clindamycin-resistant S. areus and ciprofloxacin-resistant *K. pneumoniae* strains were 500 and 250 µg/ml, respectively.

The survival percentage of *S. areus* and *K. pneumoniae* decreased to 48.05% and 11.6% respectively after treatment with 250 µg/ml C-dots. However, the viability of bacteria decreased to 3.8% and 2.5% at the concentration of 500 µg/ml.

Conclusions: The results show that by producing antibacterial drugs at the nanoscale, C-dots are a promising new approach to improve the effectiveness of treating infections caused by antibiotic-resistant bacterial strains.

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INTRODUCTION

Antibacterial treatment has become a major challenge due to the diversity of pathogenic microorganisms and their rapid mutation (1). Despite the efforts of scientists to develop new antibiotics, the rapid development of antimicrobial resistance (AMR) has led to a major global health crisis due to the lack of approval and slow development of most new drugs. Therefore, there is an urgent need for new treatments (2).

In the last few years, a wide range of

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nanomaterials with inherent biological properties has been used to combat infectious diseases caused by pathogenic bacteria (3). According to the development of nanomedicine, carbon dots (C-dots) in particular can be used to treat bacterial infections due to their high solubility, good biocompatibility, low toxicity and easy surface functionalization (3,4). C-dots are readily synthesized from a variety of carbon sources, including spermidine (5), gentamicin sulfate (6), vitamin C (7), cigarette smoke (8), bacteria, and citric acid (9) and plants (10) by various methods including chemical ablation, electrochemical carbonization, laser ablation, microwave irradiation, and hydrothermal treatment. The hydrothermal method is a one-step synthesis to prepare C-dots. Various C-dots synthesized as antibacterial agents can inhibit bacterial growth or directly kill bacteria in various ways such as oxidative stress, and interaction with DNA.(11). The rapid detection of pathogenic bacteria and the development of new antimicrobials can help reduce antimicrobial resistance, the main target of biomedical research at present.

Klebsiella pneumoniae and Staphylococcus aureus are among the most common pathogens isolated from hospital- aquired infections, burns, and post- operative infections (12). These multidrug-resistant (MDR) pathogens have caused serious problems in hospitals due to long-term infections. Multidrug-resistant bacteria are a growing risk factor that complicate or prevent treatment of infections, increasing medical complications and healthcare costs, particularly in hospital surgical and intensive care units. As a result, it is estimated that antimicrobial resistance (AMR) will be a major threat to public health by the end of 2050 (13). In this study, *Echium Italicum* root was used for the synthesis of C-dots, which contain antioxidant compounds such as shikonin and pyrrolizidine alkaloid. *E. italicum* seeds are also used in the treatment of diseases such as diabetes due to the presence of phytosterols (14). The results of this research show that our carbon dot nanoparticles can kill Gram-positive and negative bacteria as an approach and prevent microorganisms from developing resistance.

MATERIALS AND METHODS

Materials

Microbiological media (Muller Hinton agar; MHA, and Muller Hinton broth; MHB) were purchased from Merck (Merck KGaA, Germany). Roots of Echium Italicum were obtained in the local area, of Kermanshah Iran. *K. pneumoniae* (ATCC 25922), and *S. aureus* (ATCC 25923), were obtained from Mast (Mast Group Ltd, Merseyside, U.K). Antibiotics were purchased from Mast (Mast Group Ltd, Merseyside, U.K).

Synthesis of C-dots

Echium Italicum solid form and hydrothermal method were used to synthesize C-dot. First, 10 g of the prepared root was placed in a 100 ml Teflon-lined stainless steel laboratory autoclave and 50 cc of distilled water was added. The autoclave was then placed in an oven and heated to 200 degrees Celsius for 14 hours. It was then allowed to cool to room temperature. Filter paper is used to remove larger particulates. The prepared brown solution was then centrifuged three times at 6000 rpm for 30 minutes to remove large or agglomerated particles, and the supernatant containing C-dots was purified using a 0.2 μm membrane. Finally, the product was dialyzed using a 500 Da dialysis bag. The black powder C-dots were obtained after freeze-drying for 24 h.

Characterization of C-dots

Fluorescence and UV–visible measurements

A Varian Cary Eclipse spectrofluorometer equipped with a Xenon flash lamp was used for fluorescence measurements. Excitation and emission slit widths of 5 nm were used. All measurements were made in a 1 cm quartz cuvette. An Agilent 8453 diode array spectrophotometer was used to collect the UV-Vis spectra.

Fourier transform infrared spectroscopy (FTIR)

The FTIR method was used to determine the functional groups of the synthesized carbon dots. In this way, the stock solution of C-dots (100 mg/ml) was dried in an oven at 60°C and then ground with potassium bromide (KBr) pellets and analyzed in the spectral range of 400-4000 cm^{-1} with a resolution of 4 cm^{-1}.

Dynamic light scattering (DLS)

The particle size distribution measurements of C-dots were performed with a Zeta Seizer (Malvern Zeta Seizer Nano ZS90, UK) at room temperature. For DLS and zeta analysis, the stoke biosynthesized C-dots solution (100 mg/mL) was diluted in Millipore water in a 1:10 (v/v) proportion.

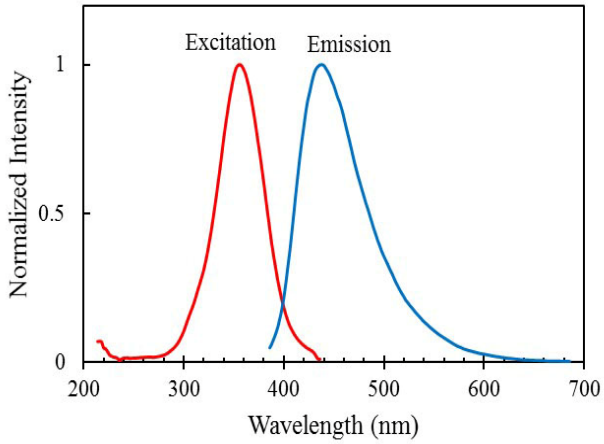

Fig.1. Fluorescence and UV–visible measurements of C-dots preparation; Inset: photograph of the obtained C-dots under visible light (left) and UV light (right).

Transmission electron microscopy (TEM)

TEM images were obtained on a Zeiss-EM10C transmission electron microscope with an accelerating voltage of 80 kV (K.N. Toosi University of Technology, Tehran, Iran). After the synthesis of C-dots, a drop of the C-dots solution was loaded on the carbon-coated copper grid and allowed to dry at 50 °C for 2h. The particle size of C-dots was determined through the image processing software Image J (Version 1.49, NIH, USA).

Antibacterial activity of C-dots

K. pneumoniae and S. areus strains were isolated from clinical specimens from Tehran Hospital and identified by standard microbiological tests. Antibiotic susceptibility was determined for all strains using the Kirby-Bayer method according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI 2021).

The antibacterial activity of C-dots was determined by the micro-broth dilution method using 96-well bottom round non-tissue culture plates according to the method published (15). Serial dilutions of C-dots solution (7.8-62.5 µg/ml) were prepared for *K. pneumoniae* ATCC 25922 (gram-negative bacteria) and *S. aureus* ATCC 35985 (gram-positive bacteria). Then, the minimum inhibitory concentration (MIC) of C-dots was evaluated by preparing dilutions (125-2000 µg/ml) for 14 clindamycin-resistant *S. aureus* strains and 14 ciprofloxacin-resistant *K. pneumoniae* strains. In brief, 50 µl of MHB medium were inoculated into wells 2 to 12. Then, 100 µl were inoculated into the first well from column 1 of C-dots, and dilution was performed. Finally, 50 µl of bacterial suspension with a concentration of 5×10^5 CFU/mL was inoculated into wells 1 to 10 and incubated at 37°C for 24 hours. Negative control (MHB medium) and positive control (MBC medium + bacteria) were also examined.

Inhibition zone assays were also determined based on previous reports (4). In brief, 30 microliters of *S. aureus* and *K. pneumoniae* strains (2x108 CFU/mL) were uniformly cultured on Mueller-Hinton agar medium and incubated at 37°C for 1 hour. A 10-mm-diameter filter paper was placed on the agar plate and incubated for 24 hours after being immersed in the carbon point solution for 1 hour. The result was recorded with a CCD camera after measuring the containment area.

Inhibition (%) =OD experiment/OD control ×100%

Statistical analysis.

T-tests were performed to analyze the data using SPSS version 23. A p-value of <0.05 was considered significant and 95% confidence intervals (CIS) were computed in the regression analyses.

RESULTS

Synthesis and characterization of C-dots

We chose *Echium Italicum* root as precursors and synthesize a carbon dot *via* a one-step hydrothermal method. Fluorescence and UV–visible measurements of C-dots are shown in Fig. 1, that the emission maximum (λem) at 455 nm when using an excitation wavelength (λex) of 360

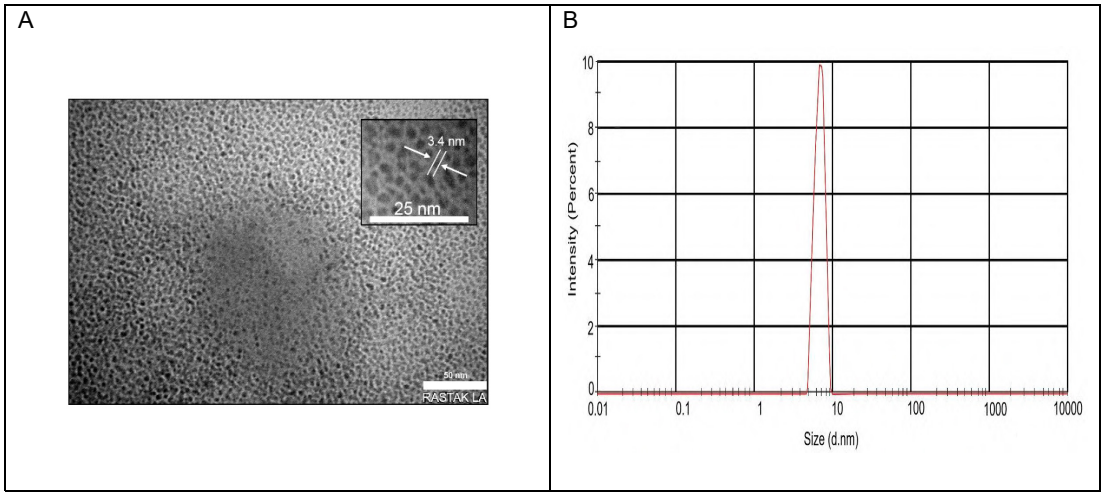

Fig. 2. (A) TEM image of the C-dots; (B) Size distributions of the C-dots.

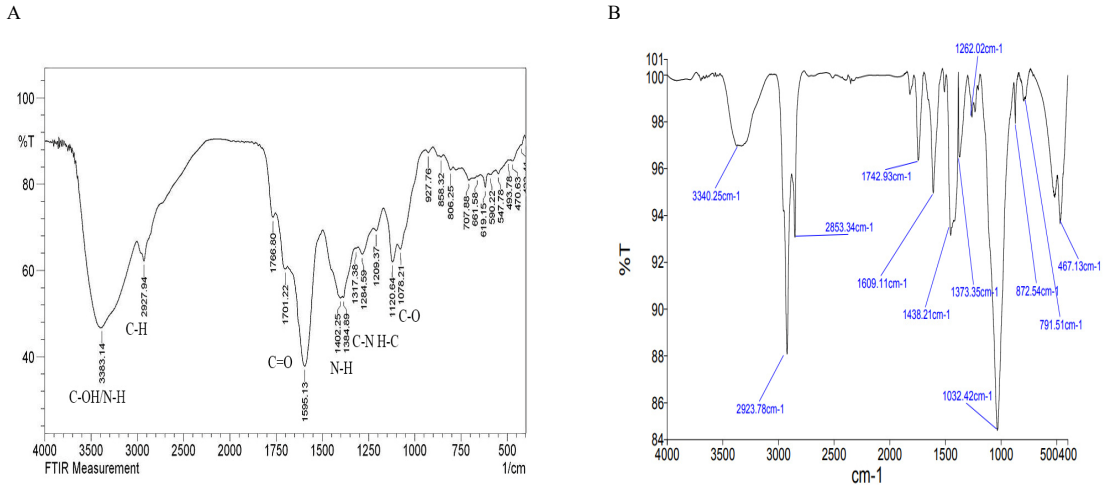

Fig. 3. FTIR spectra of C-dots and *Echium Italicum* root. important functional groups of (A) C-dots and (B) *Echium Italicum* root.

nm for C-dots. The obtained C-dots under UV light showed blue emission and were yellowish-brown under daylight. We used TEM and DLS to analyze the morphology of C-dots. TEM image reveals the size and shapes of C-dots and the C-dots morphology is nearly spherical Fig. 2A. The TEM histogram was plotted to estimate the mean C-dots diameter, which was equal to 3.4 ± 0.5 nm. The DLS data of the C-dots are shown in Fig .2B. As shown, the C-dots were Nano-sized particles around 5.43 to 10 nm in diameter. The Zeta potential of C-dots showed a positive surface charge value (+ 11.6 mV). The surface functional groups of the synthesized C-dots were studied using FTIR spectra. According to Fig. 3 A. The peaks at 3200-3550 cm^{-1} and 2800-2950 cm^{-1} can be attributed to the C-OH and C-H stretching vibrations, respectively. Stretching and bending vibrations of the C=O band of carboxyl, carbonyl, and amide groups, and peak at 1280 cm^{-1} indicate the C-NH-C group. The around 1100 cm^{-1} presents the existence of the C-O (hydroxyl, ester, epoxide, or ether) group. In general, the presence of these functional groups means that these nanomaterials have excellent water solubility without further surface modification. While Shikonin as the most active component in the *Echium Italicum* root does not contain any nitrogen atom in its structure, the appearance of the peaks related to the nitrogen-containing groups in the FTIR spectrum of the C-dots and similar peaks (1262 and 1405 cm-1) in the FTIR spectrum of the row materials extracted

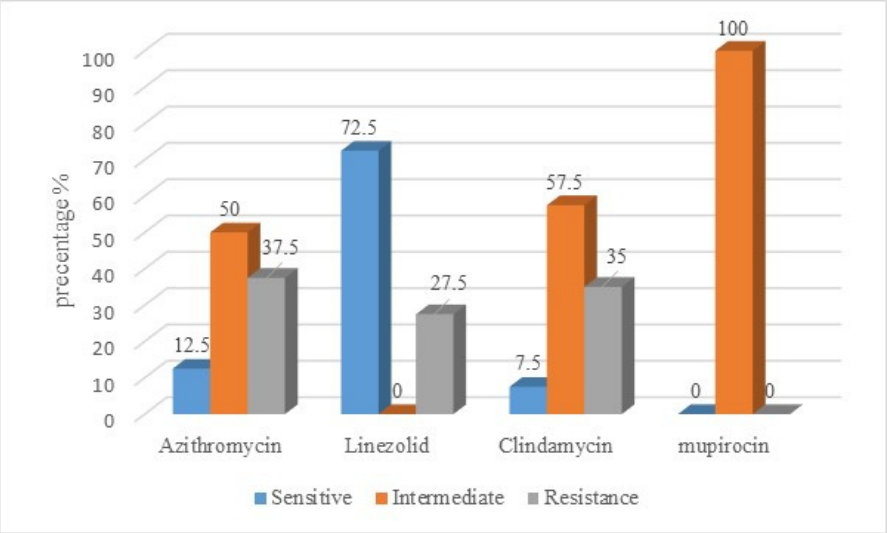

Fig. 4. The antibiotic susceptibility patterns of *S. aureus*

Table 1. The antibiotic susceptibility patterns of *Klebsiella pneumoniae* strains

Antibiotic	resistant		Semi-sensitive		Sensitive		Total
	N	%	N	%	N	%	(%)
Gentamicin(10µg)	52	49.5	2	1.9	51	48.5	105(100)
Amikacin (30 µg)	2	1.9	10	9.52	93	88.5	105(100)
Augmentin (30 µg)	30	28.5	38	36.19	37	35.2	105(100)
Ceftriaxone (30 µg)	105	100	-	-	-	-	105(100)
Ciprofloxacin (5 µg)	88	84	10	9	7	7	105(100)
Cefotaxime (30 µg)	105	100	0	0	0	0	105(100)
Ceftazidime (30 µg)	105	100	-	-	-	-	105(100)
Cefepime (30 µg)	62	59	14	13.3	29	27.6	105(100)
Cefoxitin (30 µg)	-	-	11	10.4	94	89.6	105(100)
Cephalothin (30 µg)	105	100	-	-	-	-	105(100)
Aztreonam (10 µg)	105	100	-	-	-	-	105(100)
Meropenem (10 µg)	-	-	2	1.9	103	9.09	105(100)
Imipenem (10 µg)	-	-	2	1.9	103	9.09	105(100)
Nitrofurantoin (30 µg)	7	6.6	2	1.9	96	91.4	105(100)
Cotrimoxazole (25µg)	67	63.8	10	9	28	26.6	105(100)

from the Echium Ithalicum root by ethanol Fig. 3B indicates that some nitrogen-containing active components from *Echium Italicum* root also participate in the formation of C-dots.

Antibiotic resistance K. pneumoniae and S. aureus strains

S. aureus strains to azithromycin, linezolid, and clindamycin were 37.5, 27.5, and 35%, individually. The resistance of *S. aureus* strains to antibiotics is shown in Fig. 4. The results of antibiotic susceptibility patterns of *K. pneumoniae* strains revealed all strains were resistant to antibiotics: ceftazidime, cefotaxime, ceftriaxone, and aztreonam. Also, almost all the strains were sensitive to antibiotics: imipenem, meropenem and cefoxitin. The sensitivity of *K. pneumonea* strains to antibiotics is shown in Table 1.

The antibacterial effect of C-dots on K. pneumoniae and S. aureus strains

C-dots (125-500 µg /mL) significantly reduced the growth of *S. aureus* and *K. pneumoniae* strains compared to untreated bacteria (control; $P < 0.05$). However, lower concentrations of C-dots (62.5 µg/mL) did not prevent the growth of *S. aureus*

Table.2. Antibacterial effect of C-dots on *K. pneumoniae* and *S. aureus* strains

Antimicrobial agents	*S.aureus*		*K. pneumoniae*	
	ATCC 35985	Clinical strains (n=14)	ATCC 25922	Clinical strains (n=14)
C-dots	32 µg/ml	125-500 µg/ml	62.5µg/ml	125-250 µg/ml
Clindamycin	30µg/ml	25-150 mg/ml		
Ciprofloxacin			0.5 µg/ml	16-64 µg/ml

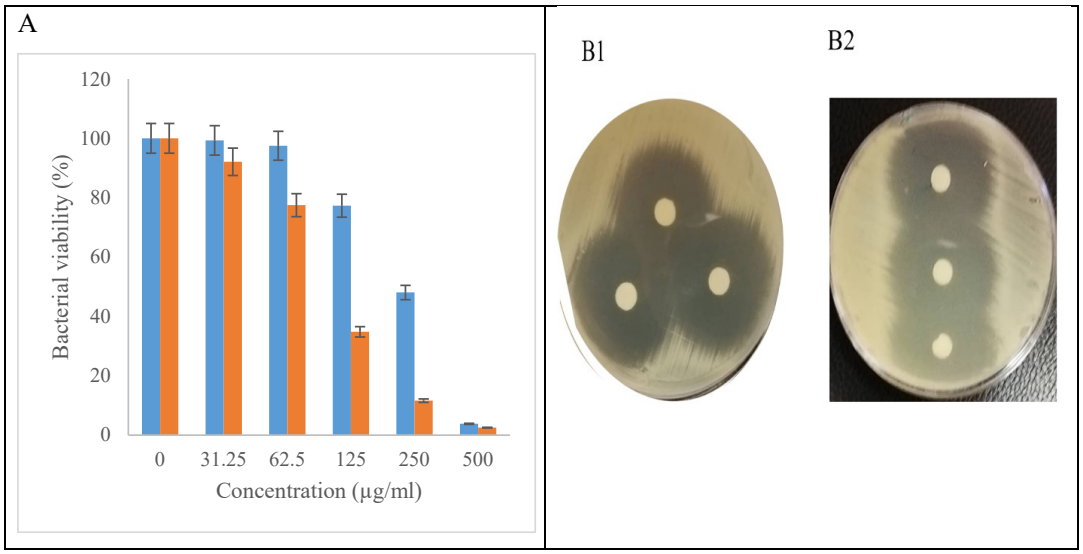

Fig. 5. Inhibitory effects of C-dots on *S. aureus* and *K. pneumoniae* growth. (A) Bacterial inhibition of the different concentration of C-dots on *S. aureus* and *K. pneumoniae* in liquid media. Inhibition zone experiments of C-dots on (B1) *S. aureus* and (B2) *K. pneumoniae*, respectively.

and *K. pneumoniae* strains (P > 0.05). Therefore, the minimum inhibitory concentration (MIC) of C-dots for clindamycin-resistant *S. aureus* and ciprofloxacin-resistant *K. pneumoniae* strains was 500 and 250 µg/ml, respectively. MIC antibiotics (clindamycin and ciprofloxacin) were also evaluated for ATCC strains and antibiotic-resistant clinical strains. A summary of the antimicrobial testing is provided in Table 2. Different concentrations of C-dots were added to the liquid medium and the OD value of bacterial growth was checked to determine the inhibitory effect of C-dots. The results in Fig. 5A showed that the survival percentage of *S. aureus* and *K. pneumoniae* strains decreased to 48.05% and 11.6%, respectively, after treatment with 250 µg/ml C-dots. However, the viability of the bacteria decreased to 3.8% and 2.5% at the concentration of 500 µg/ml. Thus, this C-dot was able to inhibit *K. pneumoniae* strains significantly compared to *S. aureus* strains (P<0.0014). Clear inhibition zones were observed on the bacterial plates (*K. pneumoniae* and S. aureus) around the filter paper treated with C-dots, as shown in Fig. 5 B1 and B2.

DISCUSSION

In the 21st century, antimicrobial resistance is becoming the most important public health issue. As antibiotic-resistant bacteria become more common, many antibiotics are becoming ineffective or less effective. The issue of resistant *S. aureus* and *K. pneumoniae* as the predominant pathogens in nosocomial infections has been of great importance for the last two decades (16).

The use of nanoparticles is increasingly being proposed because of their comparable size to the microbial system and their ability to act directly on the bacterial cell wall. NPs have different antibacterial mechanisms, so bacterial resistance to NPs is less likely than to antibiotics (17).

C-dots have received much attention because of their many properties (18). One of the most important advantages of C-dots is their functionality and surface charge, which are extremely useful for their antibacterial effects. The electrostatic interaction of the C-dots with the bacterial cell is strongly influenced by the surface charge (19). It has been reported that C-dots with

a positive surface charge have good antibacterial effects, while carbon dots with a negative charge are bacteriostatic (20).

In this study, we showed that C-dot had a positive surface charge. Therefore, the nature and surface charge of C-dots can strongly influence their interaction with the bacterial cell membrane. Ye et al. showed that C-dots synthesized by a hydrothermal method from p-phenyl-enediamine had bactericidal properties against *S. aureus* and *E. coli* strains, which was dependent on their surface charge. Since C-dots have a positive charge and the cell walls of both *E. coli* and *S. aureus* have negative charges, the antibacterial effect of C-dots was determined after the interaction by changing the bacterial membrane potential from negative to positive. The inhibitory effect of C-dots on *S. aureus* was more effective because the cell surface of *S. aureus* was more negative than that of *E. coli*. The MIC of the synthesized C-dots against *S. aureus* and *E. coli* were 2 µg/mL and 30 µg/mL, respectively,(21) In another study, C-dots were synthesized by hydrothermal method from tartaric acid and m-aminophenol, which had different positive surface charges. These C-dots at a concentration of 250 mg/ml showed more than 99% antibacterial effect, which was due to the electrostatic interaction between the positive charge of C-dots and the cell wall of *S. aureus*. (11). It was our C-dot at a concentration of 500 µg/ml that was able to prevent more than 96% of bacterial growth.

Because the porins on the bacterial membrane are nano-sized, the shape and size of the C-dots play an important role in their antibacterial effectiveness. Therefore, C-dots penetrate the bacterial cell wall, releasing the cells' intracellular components. C-dots lead to cytoplasmic leakage through complex mechanisms including ROS production, disruption of cell structure, fragmentation and densification of genomic DNA, thereby inhibiting bacterial growth or causing bacterial death (22,23). In the research, Su et al. showed that GQDs with smaller side sizes have a better bactericidal effect against *E. coli* compared to GQDs with large side sizes. This is because GQDs with smaller sizes can more easily penetrate the bacterial membrane. (24). Therefore, it can be said that the amount of damage to the bacterial cell increases as the size decreases and the reaction between the nanoparticle and the cell increases.

In our study, the antimicrobial activity of C-dots was investigated on *S. aureus* (ATCC 35985) and *K. pneumoniae* (ATCC 25922), and several ciprofloxacin-resistant *K. pneumoniae* and clindamycin-resistant *S. aureus* strains. The specific inhibitory effects of C-dots on the proliferation of clindamycin-resistant *S. aureus* and ciprofloxacin-resistant *K. pneumoniae* strains confirmed that C-dots has excellent bactericidal activity against *K. pneumoniae*. It also significantly inhibited *S. areus* at a concentration of 500 µg/ml. Furthermore, C-dot showed better inhibition of *K. pneumoniae* growth than that of Staphylococcus aureus. On the other hand, C-dots at a concentration of 250 µg/mL were able to inhibit more than 80% of *K. pneumoniae*, which is better than the antibacterial effect of 73% of the C-dot drugs reported by Liu et al. These results showed that C-dots have a good antibacterial effect at a concentration of >250 µg/ml. This C-dots are likely to act by generating ROS and fragmenting genomic DNA, thus inhibiting bacterial growth or causing their death. Zhenhui Kang et al showed that C-dots extracted from cigarette smoke have broad-spectrum antimicrobial activities that inhibit both Gram-negative and Gram-positive pathogen strains at a concentration of 1000 µg/ml. They showed that disrupting the double-helix structure of DNA may be responsible for this antibacterial property (8). Furthermore, Yanyan Wu et al. reported that C-dots hydrothermally derived from Bifidobacterium breve (B-C-dots) disrupt *E. coli* and *S. typhimurium* biofilm matrices through extensive reactive oxygen species (ROS) production, thereby reducing the volumetric density of bacteria within the biofilm. Also, these C-dots inhibited the growth of different strains of *E. coli* and *S. typhimurium* at a concentration of 500 µg/ml, which was similar to our study (25). In general, these nanomaterials are much more effective than conventional antibiotics due to the increased level of reaction with microorganisms.

In the present study, the resistance of *S. aureus* strains to azithromycin was 37.5%, while the resistance of the strains to linezolid and clindamycin was relatively lower with 27.5 and 35%. Macrolides are a group of antibiotics that have become increasingly resistant because of their repeated use in the treatment of upper and lower respiratory tract infections. Similar results, with 34.1% of azithromycin-resistant strains, were reported in a study by Sarah E. Burr et al (26). Singla et al. reported that resistance to azithromycin and erythromycin reached 20% in

Europe, especially in France and Belgium (27). In the present experiment, there was no resistance to mupirocin. The prevalence of mupirocin resistance has been reported in France, Jordan and Greece at 5%, 2.2% and 1.6%, respectively(28–30). In other studies, 18.3% and 6% of strains were resistant to mupirocin in the Godarzi et al. and Montazeri et al. studies, respectively (31,32). The lower prevalence of mupirocin resistance in these reports is because of its local and limited use in the treatment and prevention of staphylococcal skin infections. In our study, the prevalence of clindamycin and linezolid resistance was 35% and 27.5% which was higher than the prevalence of clindamycin resistance in Canada and Australia (7%) and lower than in Greece (62.4%). similar findings were reported in Ireland (19.9%) and Iran (32.3%)(33,34). Also, Goodarzi et al., Contrary to our study, all strains were sensitive to linezolid(31). Alaa Abouel fetouh et al. found that the prevalence of linezolid resistance was 1.3% among a collection of 232 clinical staphylococcal strains(35).

The results of antibiotic susceptibility patterns in *K. pneumoniae* strains showed that all strains were resistant to ceftazidime, cefotaxime, ceftriaxone, Cephalotin, and aztreonam. Also, almost all strains were sensitive to antibiotics: imipenem, meropenem, and cefoxitin. Interestingly, the majority (91%) of *K. pneumoniae* strains were sensitive to Nitrofurantoin (Table 2), which can be due to less use of this antibiotic. Shahcheraghi reported et al. that all strains of *K. pneumoniae* (100%) were resistant to clarithromycin, while 100% strains were sensitive to amikacin. Also, the resistance percentage of *K. pneumoniae* strains to ampicillin was 78.3%, cephalothin 75%, cotrimoxazole 43.9%, ceftriaxone 32%, ciprofloxacin 30.9%, cefotaxime 24% and ampicillin 20%(36). Our findings showed that the rate of resistance to the antibiotics ceftriaxone, cephalothin, cefotaxime, ciprofloxacin, and cotrimoxazole increased significantly from 2016 to 2020 in Iran. In addition, Alcántar-Curiel et al. 2018 showed that of the 168 K. pneumonia strains, 72%, 69%, and 67.2% were resistant to ceftazidime, cefotaxime, and amikacin, respectively (37). The prevalence of drug resistance of K. pneumoniae strains in Yongjun Wu's study to amikacin (40.8%), azetronam (73.3%), ceftazidime (75.7%), ciprofloxacin (59.8%), colistin (2.9%), cefotaxime (79.2%), cefepime (72.6%), and imipenem (65.6%) were different from our data. In another study, all strains of K. pneumoniae were resistant to cefotaxime (100%) and ceftazidime (100%), and 37% of the strains were resistant to gentamicin (38). One of the reasons for the increasing resistance of *K. pneumoniae* strains to antibiotics is the emergence and spread of ESBL and carbapenemase genes, which are of major public health concern. Another reason for the emergence of antibiotic-resistant strains may be the widespread use and misuse of antibiotics (39).

CONCLUSION

C-dots with appropriate environmental compatibility are known as potential antibacterial agents that can reduce antibiotic resistance. Increasing the surface area of nanoparticles provides a greater number of reactive groups, which generally increases their activity. Therefore, nanoparticles with antibiotic activities can be much more effective than conventional antibiotics. Considering that one of the major goals of current biomedical research is the prevention of bacterial infections and multidrug resistance (MDR), the development of new antibacterial agents and rapid detection of pathogens is extremely important to achieve this goal.

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No applicable

CONFLICT OF INTERESTS

None declared

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REFERENCES

1. Chung H, Merakou C, Schaefers MM, Flett KB, Martini S, Lu R, et al. Rapid expansion and extinction of antibiotic resistance mutations during treatment of acute bacterial respiratory infections. Nat Commun. 2022;13(1). https://doi.org/10.1038/s41467-022-28188-w
2. Miethke M, Pieroni M, Weber T, Brönstrup M, Hammann P, Halby L, et al. Towards the sustainable discovery and development of new antibiotics. Nat Rev Chem [Internet]. 2021;5(10):726-49. Available from: http://dx.doi.org/10.1038/s41570-021-00313-1 https://doi.org/10.1038/s41570-021-00313-1
3. Wang Y, Jin Y, Chen W, Wang J, Chen H, Sun L, et al. Construction of nanomaterials with targeting phototherapy properties to inhibit resistant bacteria and biofilm infections. Chem Eng J [Internet]. 2019;358:74-90. Available from: https://www.sciencedirect.com/science/article/pii/S1385894718319454 https://doi.org/10.1016/j.cej.2018.10.002

4. Liang G, Shi H, Qi Y, Li J, Jing A, Liu Q, et al. Specific anti-biofilm activity of carbon quantum dots by destroying p. gingivalis biofilm related genes. Int J Nanomedicine. 2020;15:5473-89. https://doi.org/10.2147/IJN.S253416
5. Jian H-J, Wu RS, Lin TY, Li YJ, Lin HJ, Harroun SG, et al. Super-Cationic Carbon Quantum Dots Synthesized from Spermidine as an Eye Drop Formulation for Topical Treatment of Bacterial Keratitis. ACS Nano. 2017;11:6703. https://doi.org/10.1021/acsnano.7b01023
6. Li P, Liu S, Cao W, Zhang G, Yang X, Gong X, et al. Low-toxicity carbon quantum dots derived from gentamicin sulfate to combat antibiotic resistance and eradicate mature biofilms. Chem Commun. 2020;56(15):2316-9. https://doi.org/10.1039/C9CC09223D
7. Li H, Huang J, Song Y, Zhang M, Wang H, Lu F, et al. Degradable Carbon Dots with Broad-Spectrum Antibacterial Activity. ACS Appl Mater Interfaces [Internet]. 2018 Aug 15;10(32):26936-46. Available from: https://doi.org/10.1021/acsami.8b08832 https://doi.org/10.1021/acsami.8b08832
8. Song Y, Lu F, Li H, Wang H, Zhang M, Liu Y, et al. Degradable Carbon Dots from Cigarette Smoking with Broad-Spectrum Antimicrobial Activities against Drug-Resistant Bacteria. ACS Appl Bio Mater. 2018;1(6):1871-9. https://doi.org/10.1021/acsabm.8b00421
9. Wu Y, van der Mei HC, Busscher HJ, Ren Y. Enhanced bacterial killing by vancomycin in staphylococcal biofilms disrupted by novel, DMMA-modified carbon dots depends on EPS production. Colloids Surfaces B Biointerfaces [Internet]. 2020;193(March):111114. Available from: https://doi.org/10.1016/j.colsurfb.2020.111114 https://doi.org/10.1016/j.colsurfb.2020.111114
10. Lin F, Li C, Chen Z. Bacteria-derived carbon dots inhibit biofilm formation of Escherichia coli without affecting cell growth. Front Microbiol. 2018;9(FEB):1-9. https://doi.org/10.3389/fmicb.2018.00259
11. Wang H, Lu F, Ma C, Ma Y, Zhang M, Wang B, et al. Carbon dots with positive surface charge from tartaric acid and m-aminophenol for selective killing of Gram-positive bacteria. J Mater Chem B. 2021;9(1):125-30. https://doi.org/10.1039/D0TB02332A
12. Duin D Van, Paterson DL. Multidrug Resistant Bacteria in the Community : An Update. 2021;34(4):709-22. https://doi.org/10.1016/j.idc.2020.08.002
13. Neill JO'. Antimicrobial Resistance: Tackling a crisis for the health and wealth of nations The Review on Antimicrobial Resistance Chaired. 2014;(December).
14. Dresler S, Szymczak G, Wójcik M. Comparison of some secondary metabolite content in the seventeen species of the boraginaceae family. Pharm Biol. 2017;55(1):691-5. https://doi.org/10.1080/13880209.2016.1265986
15. CLSI. CLSI M100-ED29: 2021 Performance Standards for Antimicrobial Susceptibility Testing, 30th Edition. Vol. 40, Clsi. 2020. 50-51 p.
16. Custovic A, Smajlovic J, Hadzic S, Ahmetagic S, Tihic N, Hadzagic H. Epidemiological Surveillance of Bacterial Nosocomial Infections in the Surgical Intensive Care Unit. Mater Socio Medica. 2014;26(1):7. https://doi.org/10.5455/msm.2014.26.7-11
17. Zhao D, Zhang R, Liu X, Li X, Xu M, Huang X, et al. Screening of Chitosan Derivatives-Carbon Dots Based on Antibacterial Activity and Application in Anti-Staphylococcus aureus Biofilm. Int J Nanomedicine. 2022;17(February):937-52. https://doi.org/10.2147/IJN.S350739
18. Varghese M, Balachandran M. Antibacterial efficiency of carbon dots against Gram-positive and Gram-negative bacteria: A review. J Environ Chem Eng [Internet]. 2021;9(6):106821. Available from: https://doi.org/10.1016/j.jece.2021.106821 https://doi.org/10.1016/j.jece.2021.106821
19. Li P, Poon YF, Li W, Zhu HY, Yeap SH, Cao Y, et al. A polycationic antimicrobial and biocompatible hydrogel with microbe membrane suctioning ability. Nat Mater [Internet]. 2011;10(2):149-56. Available from: http://dx.doi.org/10.1038/nmat2915 https://doi.org/10.1038/nmat2915
20. Varghese M, Balachandran M. Antibacterial efficiency of carbon dots against Gram-positive and Gram-negative bacteria: A review. J Environ Chem Eng. 2021;9(6). https://doi.org/10.1016/j.jece.2021.106821
21. Ye Z, Li G, Lei J, Liu M, Jin Y, Li B. One-Step and One-Precursor Hydrothermal Synthesis of Carbon Dots with Superior Antibacterial Activity. Vol. 3, ACS Applied Bio Materials. 2020. 7095-7102 p. https://doi.org/10.1021/acsabm.0c00923
22. Li Y-J, Harroun SG, Su Y-C, Huang C-F, Unnikrishnan B, Lin H-J, et al. Synthesis of Self-Assembled Spermidine-Carbon Quantum Dots Effective against Multidrug-Resistant Bacteria. Adv Healthc Mater. 2016;5:2545. https://doi.org/10.1002/adhm.201600297
23. Bing W, Sun H, Yan Z, Ren J, Qu X. Programmed Bacteria Death Induced by Carbon Dots with Different Surface Charge. Small. 2016;12:4713. https://doi.org/10.1002/smll.201600294
24. Su S, Shelton CB. Size Dependent Antibacterial Behavior. ASME Int Mech Eng Congr Expo Proc. 2016;15:1-4.
25. Wu Y, Yang G, Mei HC Van Der, Shi L, Busscher HJ. Synergy between " Probiotic " Carbon Quantum Dots and Ciprofloxacin in Eradicating Infectious Biofilms and Their Biosafety in Mice. 2021; https://doi.org/10.3390/pharmaceutics13111809
26. Bojang E, Jafali J, Perreten V, Hart J, Harding-Esch EM, Sillah A, et al. Short-term increase in prevalence of nasopharyngeal carriage of macrolide-resistant Staphylococcus aureus following mass drug administration with azithromycin for trachoma control. BMC Microbiol. 2017;17(1):1-10. https://doi.org/10.1186/s12866-017-0982-x
27. den Heijer CDJ, van Bijnen EME, Paget WJ, Pringle M, Goossens H, Bruggeman CA, et al. Prevalence and resistance of commensal Staphylococcus aureus, including meticillin-resistant S aureus, in nine European countries: A cross-sectional study. Lancet Infect Dis [Internet]. 2013;13(5):409-15. Available from: http://dx.doi.org/10.1016/S1473-3099(13)70036-7 https://doi.org/10.1016/S1473-3099(13)70036-7
28. Aqel AA, Ibrahim A, Shehabi A. Rare occurrence of mupirocin resistance among clinical Staphylococcus isolates in Jordan. Acta Microbiol Immunol Hung. 2012;59(2):239-47. https://doi.org/10.1556/amicr.59.2012.2.8
29. Desroches M, Potier J, Laurent F, Bourrel AS, Doucet-Populaire F, Decousser JW, et al. Prevalence of mupirocin resistance among invasive coagulase-negative staphylococci and methicillin-resistan Staphylococcus aureus (MRSA) in France: Emergence of a mupirocin-resistant MRSA clone harbouring mupA. J Antimicrob Chemother. 2013;68(8):1714-7. https://doi.org/10.1093/jac/dkt085
30. Petinaki E, Spiliopoulou I, Kontos F, Maniati M, Bersos Z, Stakias N, et al. Clonal dissemination of mupirocin-resistant staphylococci in Greek hospitals. J Antimicrob

Chemother. 2004;53(1):105-8. https://doi.org/10.1093/jac/dkh028
31. Abbasi-Montazeri E, Khosravi AD, Feizabadi MM, Goodarzi H, Khoramrooz SS, Mirzaii M, et al. The prevalence of methicillin resistant Staphylococcus aureus (MRSA) isolates with high-level mupirocin resistance from patients and personnel in a burn center. Burns [Internet]. 2013;39(4):650-4. Available from: http://dx.doi.org/10.1016/j.burns.2013.02.005 https://doi.org/10.1016/j.burns.2013.02.005
32. Goudarzi M, Kobayashi N, Dadashi M, Pantůček R, Nasiri MJ, Fazeli M, et al. Prevalence, Genetic Diversity, and Temporary Shifts of Inducible Clindamycin Resistance Staphylococcus aureus Clones in Tehran, Iran: A Molecular-Epidemiological Analysis From 2013 to 2018. Front Microbiol. 2020;11(April):1-18. https://doi.org/10.3389/fmicb.2020.00663
33. Castanheira M, Watters AA, Bell JM, Turnidge JD, Jones RN. Fusidic acid resistance rates and prevalence of resistance mechanisms among Staphylococcus spp. isolated in North America and Australia, 2007-2008. Antimicrob Agents Chemother. 2010;54(9):3614-7. https://doi.org/10.1128/AAC.01390-09
34. Rezaie Keikhaie K, Sargazi A, Hassansnhahian M, Shahi Z. Detection of Intracellular Adhesion (ica) and Biofilm Formation Genes in Staphylococcus aureus Isolates from Clinical Samples. Res Mol Med. 2017;5(1):40-3. https://doi.org/10.29252/rmm.5.1.40
35. Kamali E, Jamali A, Ardebili A, Ezadi F, Mohebbi A. Evaluation of antimicrobial resistance, biofilm forming potential, and the presence of biofilm-related genes among clinical isolates of Pseudomonas aeruginosa. BMC Res Notes [Internet]. 2020;13(1):4-9. Available from: https://doi.org/10.1186/s13104-020-4890-z https://doi.org/10.1186/s13104-020-4890-z
36. Ayatollahi J, Sharifyazdi M, Fadakarfard R, Hossein S. Antibiotic resistance pattern of Klebsiella pneumoniae in obtained samples from Ziaee Hospital of Ardakan , Yazd, Iran during 2016 to 2017. 2020;02:32-6. https://doi.org/10.53986/ibjm.2020.0008
37. Alcántar-curiel MD, Ledezma-escalante CA, Jarillo-quijada MD, Gayosso-vázquez C, Morf R, Rodr E, et al. Association of Antibiotic Resistance , Cell Adherence, and Biofilm Production with the Endemicity of Nosocomial Klebsiella pneumoniae. 2018;2018. https://doi.org/10.1155/2018/7012958
38. Ahanjan M, Naderi F, Solimanii A, Practitioner G. Prevalence of Beta-lactamases Genes and Antibiotic Resistance Pattern of Klebsiella pneumoniae Isolated from Teaching hospitals, Sari, Iran, 2014. Journal of Mazandaran University of Medical Sciences. 2017 Jun 10;27(149):79-87.
39. Queenan AM, Bush K. Carbapenemases : the Versatile -Lactamases. 2007;20(3):440-58. https://doi.org/10.1128/CMR.00001-07