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RESEARCH ARTICLE

Comparing the production of carbon dots synthesized from *Lactobacillus acidophilus* and *Bifidobacterium bifidum* and investigating their antibacterial effects

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ABSTRACT

Objective(s): *Klebsiella pneumoniae* is a significant opportunistic bacterial pathogen, responsible for over 70% of human infections. The development of carbapenem resistance is considered a major risk to public health.

Methods: Cultivation of *Klebsiella pneumonia* isolates (100 samples) for phenotypic identification. Drug sensitivity was evaluated by disc diffusion method, and carbapenemase-producing isolates were identified by the mCIM and eCIM methods. *Lactobacillus acidophilus* and *Bifidobacterium bifidum* were cultured and carbon dots were synthesized by hydrothermal method. The physicochemical properties of the carbon dots were investigated and their antibacterial activity against *Klebsiella pneumonia* isolates was determined.

Results: After identifying *Klebsiella pneumonia* isolates, 70 carbapenem-resistant isolates were found among the samples. Of these, 41% were serine carbapenemase and 29% were metallo-beta-lactamase. The minimum inhibitory concentration (MIC) for synthesized carbon dots was observed to be around 50 mg/mL.

Conclusions: Due to their beneficial properties, carbon dots can be used as an antimicrobial agent to treat antibiotic-resistant infectious diseases. This group of nanoparticles exhibits high activity and can be proposed as a new strategy to combat resistant infections.

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INTRODUCTION

Klebsiella pneumoniae is a member of the Enterobacteriaceae family is frequently present in the human gastrointestinal tract and surrounding areas, particularly in hospital settings. As a significant opportunistic bacterial pathogen, this bacterium causes over 70% of human infections in various body sites. It can cause severe drug-resistant infections if not treated properly (1). Carbapenem resistance is a significant issue for this bacterium. The emergence of this drug resistance class has made it even more critical due to limited treatment options, various drug complications, and a high mortality rate associated with infections(2).

Due to the increase in antibiotic-resistant micro-organisms, the discovery of novel antibacterial agents is a time-consuming and difficult process. Therefore, alternative strategies to combat drug resistance are necessary. One proposed solution

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is the use of nanotechnology and the synthesis of Carbon Dots (C-dots)(3). Carbon dots have gained significant attention in various biomedical applications due to their unique optical properties, small size (less than 10 nm), good biocompatibility, limited toxicity, affordable synthesis cost and ease of modification (4,5). Nanoparticles have different structures and properties due to different synthesis processes and precursors. These particles can be readily passivated or functionalized with organic groups, which is crucial for the distinctive properties of carbon dots. Carbon dots are a promising method for specifically inactivating various bacterial species. An effective strategy to combat resistant pathogens is the photodynamic inactivation of bacteria using photosensitizers. This method relies on the production of highly reactive oxygen species (ROS) that can inactivate bacterial cells through various means, including cell membrane destruction and DNA damage. Carbon dots are therefore a promising class of nanomaterials for the targeted deactivation of various bacterial species through the generation of highly active oxygen species (5,6,7). The research group demonstrated that C-dots' antibacterial effects are primarily due to the generation of reactive oxygen species (ROS). As they spread and become infected, the pathogens become more susceptible to antibiotics. C-dots synthesized through methods such as hydrothermal can inherit properties similar to those of their carbon sources. Therefore, C-dots can be used synergistically with antibiotics to treat various bacterial infections. Reducing the volume density of resistant pathogenic bacteria caused by exposure to these particles can enhance the effectiveness of antibiotics in killing bacteria. Suitable sources for the synthesis of antibacterial carbon dots are therefore antibiotics, medicinal plants and probiotic bacteria (3,8,9).

This study introduces a new type of C-dots that can be easily prepared using probiotic bacteria, *Lactobacillus acidophilus* and *Bifidobacterium bifidum*, by a one-step hydrothermal method. The antibacterial effects of these C-dots on carbapenem-resistant *Klebsiella pneumoniae* isolates are evaluated without any chemical or physical modification.

MATERIALS AND METHODS

Growth of bacterial isolates

One hundred *Klebsiella pneumoniae* isolates from various body sites were available in the university bacteriology laboratory archive. All strains were cultured aerobically for 24 hours on blood agar culture medium with 5% sheep blood at 35±2°C(10).

Lactobacillus acidophilus (*L. acidophilus*) and *Bifidobacterium bifidum* (*B. bifidum*) were cultured anaerobically on a De Man, Rogosa and Sharpe (MRS) medium agar plate at 35±2°C overnight, as probiotic bacteria(9).

Detection of bacteria

The *Klebsiella pneumoniae* isolates were identified using colony morphology, Gram stain, and biochemical tests including TSI, Citrate, MR, VP, SIM, and Urea (10).

We performed phenotypic identification using Gram stain, catalase and oxidase tests to identify probiotic bacteria. Subsequently, we performed molecular detection by first extracting the bacteria using the boiling method. We determined the quality of the extracted sample using a 1% agarose gel (11). The polymerase chain reaction (PCR) was performed using specific primers prepared by Pishgam, which include for *Lactobacillus acidophilus,* Acidfor (AGCGAGCTGAACCAACAGAT), Acidrev (AGGCCGTTACCCTACCAACT) and *Bifidobacterium bifidum*, *16SrRNA*-F (TCGCTAGTAATCGCGGATCA), *16SrRNA*-R (GACGGGCGGTGTGTACAAG). The primer sequences were identified by conducting a BLAST search on the NCBI GenBank database to find the closest match. Amplification was performed using a thermal cycler (Bio-Rad, MJ Mini, USA). The final reaction mixture comprises 20 µl, consisting of 10 µl of master mix (provided by Amplicon Company), 1 µl of each primer (forward, reverse), 6 µl of sterile distilled water, and 2 µl of DNA. The DNA fragments for bacteria were amplified using the following protocol: The PCR reaction was initiated with an initial denaturation step at 94°C for 3 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 60°C for 20 seconds, and extension at 72°C for 20 seconds. The reaction was completed with a final extension step at 72°C for 5 minutes. To analyze the PCR products, 2µl aliquots were electrophoresed in 1.5% agarose gels in TBE 1X buffer. The gels were then stained with GelRed and gene sequences were identified based on size and charge using visual techniques (12,13).

Antimicrobial Susceptibility test (disc diffusion method)

The antimicrobial susceptibility of each isolate was tested following the Clinical & Laboratory Standards Institute (CLSI) guidelines. Fresh cultures of stock *Klebsiella pneumoniae* isolates were prepared and incubated at 35±2°C overnight. Then, a turbidity equal to 0.5 McFarland (10^8cfu/mL) was prepared from one colony of isolates and cultivated on Mueller Hinton Agar (MHA) medium in the form of grass. Antibiotic discs were placed on the medium at regular intervals. The incubation process is carried out at a temperature of 35±2°C for a duration of 16-18 hours. Following the completion of the incubation, the plates are read, and the results are reported (14,15).

Detection of carbapenemase-producing Klebsiella pneumoniae

The modified carbapenem inactivation method (mCIM) and EDTA carbapenem inactivation method (eCIM), which are new types, were used to test bacterial isolates for the presence of carbapenemase enzymes following CLSI guidelines (16).

Synthesis of carbon dots

A single colony of bacteria was inoculated into 10 mL of MRS broth and incubated anaerobically overnight at 35±2°C. The pre-culture was transferred to fresh MRS broth (200 mL) and incubated for 18 hours under the same temperature conditions. The bacterial cultures were poured into sterile tubes and centrifuged at 5000×g for 5 minutes. The pellets obtained were washed twice with sterile demineralised water (DW) and then re-suspended in 40 mL of demineralised water. After washing, place the bacterial suspension (approximately 30-40 ml) in a 100 mL Teflon-lined stainless-steel autoclave and heat it in a 200°C oven for 24 hours. To remove large carbon dots from the dark-brown liquid, centrifuge the liquid at 15000xg for 15 minutes and separate the supernatant. Filter the supernatant through a 0.22 µm filter membrane. Store the carbon dots derived from bacteria in the refrigerator until freeze-drying overnight (3,9). The synthesized C-dots were weighed and prepared for physico-chemical characterization, as shown in Fig. 1.

Examining the characteristics of carbon dot

C-dots were examined via high-resolution transmission electron microscopy (HR-TEM) to study material microstructure at atomic scales. Fourier transform infrared (FTIR) spectra, commonly used for compound identification, were utilized in the 400-4000 cm^{-1} spectral range with a 4 cm^{-1} resolution. Photoluminescence spectroscopy (PL) and Ultraviolet–visible Spectroscopy (UV–vis) were used to record the fluorescence and absorption spectra of C-dots, respectively (3,9,17).

Antibacterial activity of C-dots synthesized from Lactobacillus acidophilus and Bifidobacterium bifidum

This method employs a 96-well microplate and C-dots, with an initial concentration of 200 mg/ml, against *Klebsiella pneumoniae* isolates that are resistant to carbapenems. The suspension has a turbidity of 0.5 McFarland (10^8cfu/mL)

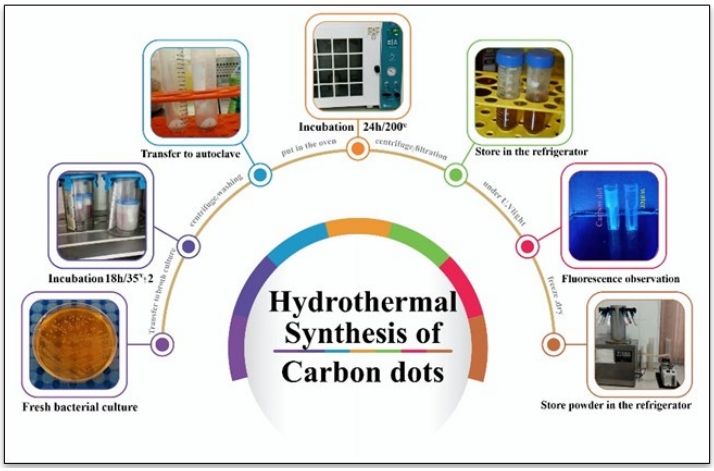

Fig. 1. Steps of carbon dot synthesis from bacteria.

and is prepared by diluting the suspension (1/20) to10^6cfu/mL. The culture medium of Nutrient Broth is also distributed inside the well. After incubating overnight at 35 ± 2 °C, the optical absorbance (OD) was measured using an Eliza reader at a wavelength of 620 nm. The minimum inhibitory concentration (MIC) is defined as the lowest concentration of antibacterial agent that inhibits bacterial growth. To determine the Minimal Bactericidal Concentration (MBC) of an antibacterial agent, bacterial suspensions are inoculated onto Mueller Hinton agar medium and incubated overnight at 35±2°C. The concentration at which no colonies are formed is recorded as the MBC (9,18).

Statistical analysis

All statistical analyses were performed using Excel 2016 and were used for data analysis.

RESULTS

Isolation and identification of bacteria

All isolates of *Klebsiella pneumoniae* grew well on blood agar culture medium. Phenotypic identification revealed gram-negative bacilli with the following biochemical test results: IMVIC (-, -, +, +), TSI (acid/acid), and positive for urea agar. *L. acidophilus and B. bifidum* grew well on MRS agar. They appeared as gram-positive coccobacilli in morphology (gram staining) and tested negative for catalase and oxidase. The samples exhibited suitable smear bands on the 1% agarose gel for molecular diagnosis, confirming the accuracy of the extraction process. The PCR product results are also visible as distinct bands on a 1.5% agarose gel using the 50bp and 100bp markers (Fig. 2).

Antimicrobial Susceptibility test

Seventy out of 100 *Klebsiella pneumoniae* isolates were found to be resistant to carbapenems. Of these, 62% and 63% were resistant to imipenem (10µg) and meropenem (10µg), respectively. Fig. 3 shows a graphical representation of all Antibiotic susceptibility test results.

Detection of carbapenemase-producing Klebsiella pneumoniae

The diagram below (Figs. 4, 5) shows the frequency of production of various carbapenemase resistances in *Klebsiella pneumoniae* isolates, including serine carbapenemase and metallo-carbapenemase.

Physico-Chemical Characterization of carbon dots

The HR-TEM analysis revealed that *L. acidophilus* and *B. bifidum* have spherical shapes with average diameters of 5.83±2.035 nm and 3.32±0.592 nm, respectively, as shown in Fig. 6a and 6b.The FTIR spectra of synthetized C-dots and source bacteria of *L. acidophilus* and *B. bifidum* demonstrated absorption bonds at 3464, 3411 cm^{-1} and 3506, 3410 cm^{-1} which were attributed to the stretching vibrations of v (OH/N–H), respectively (they indicate hydrogen bonding, and this band confirms the presence of hydrate, hydroxyl, and ammonium). The absorption bonds at 2927, 2934 cm^{-1} and 2852-2924, 2890-2943 cm^{-1} which were attributed to the stretching vibrations of v(C–H), respectively. Also, the presence of peaks in these ranges indicates the vibrational characteristics of v(C-OH) and v(C-H) and in source bacteria absorption bond at 2692 cm^{-1} is carboxyl acid overtone and combination bands. Carbonization of bacteria to carbon dots resulted in a significant band in the absorption ratio of Amide I (1667 cm^{-1} and 1649 cm^{-1} due to C=O stretching vibrations) that was not seen in the source bacteria and it attested to the presence of proteins, In Fig. 6c and 6d (19,20). In Fig. 6e, the UV/vis spectrum of *L. acidophilus* and *B. bifidum* C-dot samples shows three absorption bands at 260, 346, 391 nm and 263, 348 and 393 nm, respectively, which can be attributed to electron transitions π-π* (C=C), n-π*

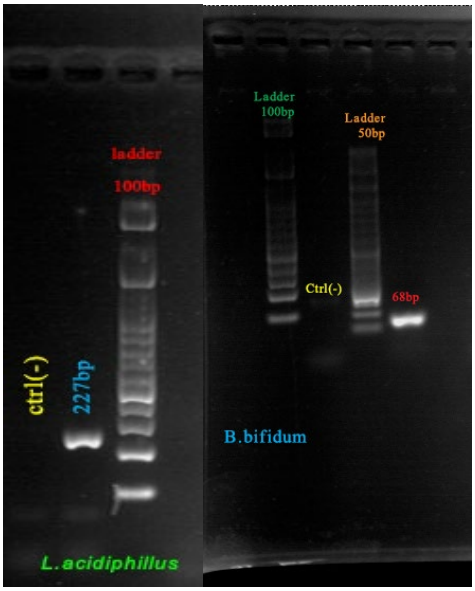

Fig. 2. Electrophoretic patterns of amplified *L. acidophilus* DNA (227bp) and *B. bifidum* DNA (68bp) on the 1.5 % agarose gel with 50 (bp) and 100 (bp) markers.

(C= O) and n-π* attributed other factor groups on sample. In Fig. 6f, the 2D PL spectrum of samples, the prominent characteristic of C-dots, which can be seen by increasing the red-shift absorption wavelength in the emission spectrum. According to the figures, as the absorption wavelength increases from 260 - 350 nm and 263- 353 nm, respectively, the emission spectrum gradually shifts to higher wavelengths along with a decrease in PL intensity (red shift) (3,9,21-23).

Antibacterial activity of carbon dots

In determining the Minimum inhibitory concentration (MIC) in both synthesized C-dots, the highest number was related to the concentration of 50 mg/ml. Especially in the C-dot synthesized

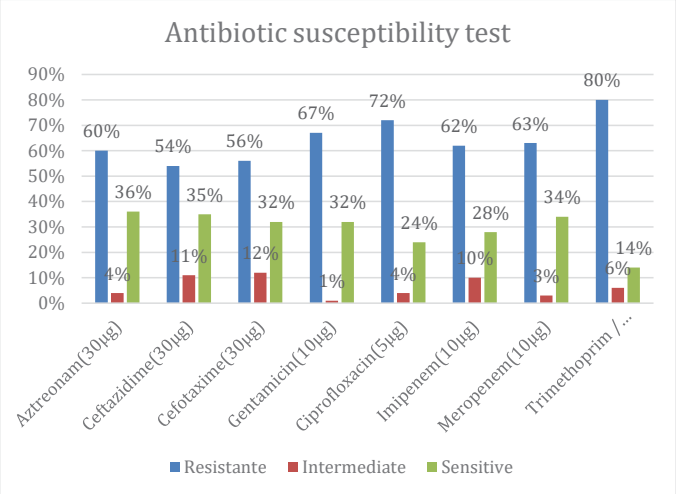

Fig. 3. Antibiotic susceptibility pattern of *K. pneumonia* isolates (100 isolates)

Fig. 4. Carbapenemase detection results of *K. pneumonia* isolates with mCIM and eCIM.

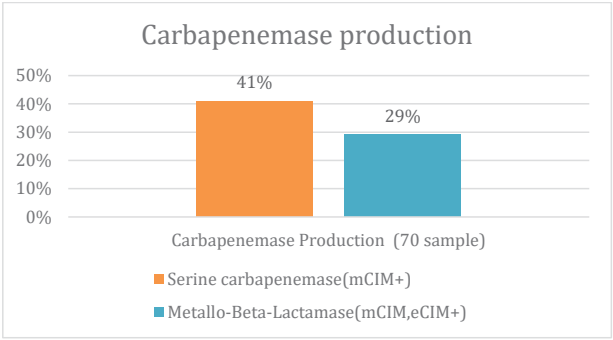

Fig. 5. Frequency chart of *K. pneumonia* isolates producing carbapenemase.

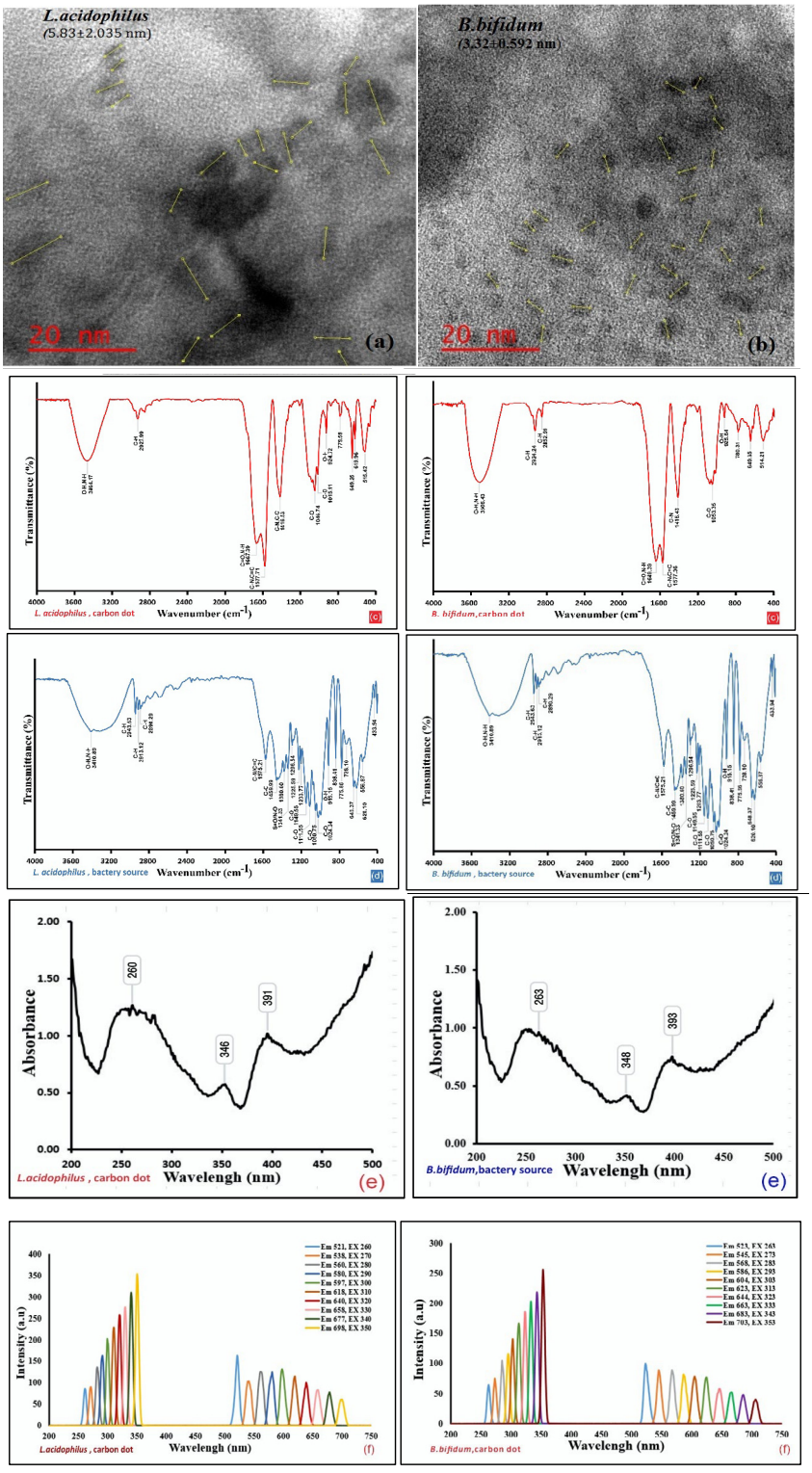

Fig. 6. (a, b) HR-TEM image and the corresponding size distribution histogram of carbon dots. (c) FTIR spectra of carbon dots derived bacteria (red), (d) FTIR spectra of source bacteria (blue). (e) Absorption UV/vis spectrum. (f) 2D emission-absorption PL spectrum.

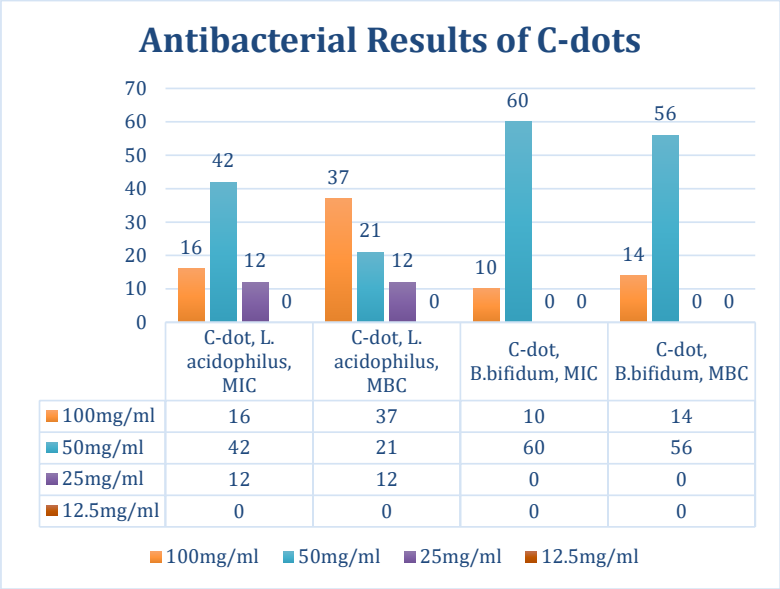

Fig. 7. The pattern of determining the Minimum inhibitory concentration (MIC) and Minimal Bactericidal Concentration (MBC) of synthesized C-dots.

from *B. bifidum*, this amount was observed in about 60/70 samples (85%), but in the C-dot synthesized from *L. acidophilus*, it was about 42/70 samples (60%). All the obtained results along with the Minimal Bactericidal Concentration (MBC) are shown in the diagram of Fig. 7.

DISCUSSION

Carbapenemase resistance caused by bacteria, including *Klebsiella pneumoniae*, is a significant problem for health services worldwide, including Iran. The emergence and global spread of this resistance is a matter of concern. *Klebsiella pneumoniae* is a member of the Enterobacteriaceae family and is commonly found in the digestive system and surrounding environment, particularly in hospital environments. Carbapenems are frequently prescribed as the primary antibiotic treatment for severe infections caused by multidrug-resistant *Klebsiella pneumoniae*. Given the rise of antibiotic resistance, it is crucial to identify effective methods to inhibit and control the growth of resistant bacteria (24,25). Therefore, there is an increasing number of experiments aimed at finding non-antibiotic treatment options. The replacement of antibiotics with suitable new drugs that have antibacterial properties requires extensive research and time. Currently, there is significant interest in the inherent antibacterial properties of nanoparticles (NPs). The bacteriostatic and bactericidal efficacy of these particles against a wide range of bacteria has been proven in many studies. The small size of NPs (measured in nanometers) allows them to directly interact with bacterial cell walls, making them effective agents in combating bacterial infections. Nanoparticles exhibit lower bacterial resistance than antibiotics due to their varied antibacterial mechanisms (26). Although some materials, such as metals, have good antibacterial properties, it is essential to know that reducing the particle size to nanometres can increase their effectiveness. Nanoparticles have specific bactericidal mechanisms that contribute to their ability to kill bacteria. The antibacterial mechanism of nanoparticles is not fully understood, and it can vary between different types of nanoparticles. These differences can be attributed to the physical structure of nanoparticles (27). Carbon dots are a type of nanoparticle that has garnered significant attention from researchers. They are synthesized from various sources, including bacteria such as probiotics, and are extremely small (9). Carbon dots exhibit a photodynamic antibacterial mode, making it an effective strategy to counteract bacterial resistance. They can impact bacterial metabolism and infiltrate and damage bacterial cell membranes by adhering to them. During the antimicrobial process of C-dots, smaller particle

size allows for better penetration. Additionally, the surface functional groups and charges of these particles can affect their antimicrobial activity. It should be noted that C-dots can also produce reactive oxygen species (ROS) which can destroy bacterial membranes. C-dots have good biocompatibility, but their production of ROS could be toxic to cells. Therefore, it is recommended to expose photo-excited C-dots to pathogenic bacteria. Additionally, functionalization of C-dots should be targeted towards bacteria. Polymer materials could be composited with carbon dots, according to suggestions. The polymer can interact with bacteria, leading to the carbon dots being released at the infection site. This can prevent tissue cells from the damaging effects of ROS (3,9,24). However, carbon dots show slightly stronger antibacterial effects against Gram-positive bacterial strains than against Gram-negative bacteria. This is due to the fact that Gram-negative bacteria possess internal and external lipid membranes, including lipids, proteins, and lipopolysaccharides, which hinder the entry of carbon dots into the cells, thereby reducing their antibacterial activity (28,29). Apart from their antibacterial activity, C-dots are a new type of fluorescent nanomaterials with excellent luminescence. They can be labeled easily and inexpensively for selective bio-labeling of bacteria and intracellular organelles. This makes them useful for a wide range of purposes, including biological applications and as fluorescent carriers for targeted drug delivery (30,31,32,33). Especially labeling them with probiotics for drug delivery applications that has been proposed recently (28,34). As described, C-dots possess beneficial properties that can be attained through proper and precise synthesis, including antibacterial activity. Finally, extensive studies have shown that C-dots have the potential to be effective antibacterial agents, reducing antibiotic resistance in bacteria. They can be compared favorably to other antibacterial agents. These nanoparticles are non-toxic, inexpensive, and easily manipulated to enhance performance. It also reduces the likelihood of bacterial resistance. Additionally, their antibacterial efficiency can be improved with proper surface function. But the exact mechanisms behind their antibacterial activity are still unclear (6,35). Extensive studies are necessary to discover the appropriate synthesis of C-dots at high scales with precise size and controllable surface properties. The properties of the synthesized C-dots were almost identical, with the only difference being the size of the carbon dot, which was larger in *L. acidophilus*. The only discernible difference between the two synthesized C-dots was their inhibitory activity. Specifically, the C-dots synthesized from *B. bifidum* exhibited inhibitory activity in approximately 80% of the samples at a lower concentration than the other C-dots. The smaller size of the C-dot may be a reason for its enhanced antibacterial effect. Additionally, C-dots act as auxiliary agents in antibacterial or anti-biofilm activity. Therefore, it is recommended to use them in conjunction with other antibacterial substances, such as antibiotics, to observe their synergistic effect. It should be investigated whether they will play a significant role in treating infections that are resistant to treatment (9,36).

CONCLUSION

The study proposes new solutions for eliminating treatment-resistant bacteria and controlling/preventing their spread. C-dots synthesized from a bacterial source exhibit antibacterial property, which can be utilized to combat these dangerous organisms to some extent. To achieve higher goals, extensive studies are necessary to identify C-dots and determine how to modify them to obtain more useful properties.

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No applicable.

CONFLICT OF INTERESTS

None declared.

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REFERENCES

1. Shi L, Feng J, Zhan Z, Zhao Y, Zhou H, Mao H, et al. Comparative analysis of blaKPC-2-and rmtB-carrying IncFII-family pKPC-LK30/pHN7A8 hybrid plasmids from klebsiella pneumoniae CG258 strains disseminated among multiple Chinese hospitals. Infect Drug Resist. 2018;11:1783-93. https://doi.org/10.2147/IDR.S171953
2. Chen CC, Lai CC, Huang HL, Huang WY, Toh HS, Weng TC, et al. Antimicrobial activity of lactobacillus species against carbapenem-resistant enterobacteriaceae. Front Microbiol. 2019;10(APR):1-10. https://doi.org/10.3389/fmicb.2019.00789
3. 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). https://doi.org/10.3389/fmicb.2018.00259
4. Singh I, Arora R, Dhiman H, Pahwa R. Carbon quantum

dots: Synthesis, characterization and biomedical applications. Turkish J Pharm Sci. 2018;15(2):219-30. https://doi.org/10.4274/tjps.63497
5. Dong X, Liang W, Meziani MJ, Sun YP, Yang L. Carbon dots as potent antimicrobial agents. Theranostics. 2020;10(2):671-86. https://doi.org/10.7150/thno.39863
6. Li P, Sun L, Xue S, Qu D, An L, Wang X, et al. Recent advances of carbon dots as new antimicrobial agents. SmartMat. 2022;3(2):226-48. https://doi.org/10.1002/smm2.1131
7. Ghirardello M, Ramos-Soriano J, Galan MC. Carbon dots as an emergent class of antimicrobial agents. Nanomaterials. 2021;11(8):1-24. https://doi.org/10.3390/nano11081877
8. Barzegari A, Kheyrolahzadeh K, Mahdi S, Khatibi H, Sharifi S, Memar MY, et al. The battle of probiotics and their derivatives against biofilms. Infection and Drug Resistance. 2020. p. 659-672. https://doi.org/10.2147/IDR.S232982
9. Wu Y, Yang G, van der Mei HC, Shi L, Busscher HJ, Ren Y. Synergy between "probiotic" carbon quantum dots and ciprofloxacin in eradicating infectious biofilms and their biosafety in mice. Pharmaceutics. 2021;13(11). https://doi.org/10.3390/pharmaceutics13111809
10. Osman EA, El-Amin N, Adrees EAE, Al-Hassan L, Mukhtar M. Comparing conventional, biochemical and genotypic methods for accurate identification of Klebsiella pneumoniae in Sudan. Access Microbiol. 2020;2(3):2-5. https://doi.org/10.1099/acmi.0.000096
11. Abdulla AA. Optimization of DNA extraction of Lactobacillus spp for identification by tuf B gene-based polymerase chain reaction. J Biol Agric Healthc. 2014;4(8):122-7.
12. Tabasco R, Paarup T, Janer C, Peláez C, Requena T. Selective enumeration and identification of mixed cultures of Streptococcus thermophilus, Lactobacillus delbrueckii subsp. bulgaricus, L. acidophilus, L. paracasei subsp. paracasei and Bifidobacterium lactis in fermented milk. Int Dairy J. 2007;17(9):1107-14. https://doi.org/10.1016/j.idairyj.2007.01.010
13. Rezania N, Rahmati P, Noorbakhsh F, Farhadyar N, Lotfali E. Investigation the effects of silver nanoparticles and gold nanoparticles on expression of bap and csu genes in biofilm formation of Acinetobacter baumannii. Iran J Microbiol. 2022;14(4):510-7. https://doi.org/10.18502/ijm.v14i4.10237
14. Tsai YM, Wang S, Chiu HC, Kao CY, Wen LL. Combination of modified carbapenem inactivation method (mCIM) and EDTA-CIM (eCIM) for phenotypic detection of carbapenemase-producing Enterobacteriaceae. BMC Microbiol. 2020;20(1):1-7. https://doi.org/10.1186/s12866-020-02010-3
15. Hudzicki J. Kirby-Bauer Disk Diffusion Susceptibility Test Protocol Author Information. Am Soc Microbiol [Internet]. 2012;(December 2009):1-13.
16. Methods G. Detection of the Klebsiella pneumoniae carbapenemase (KPC) in K.pneumoniae Isolated from the Clinical Samples by the Phenotypic and Genotypic Methods. Iran J Pathol. 2012;353900(5865):199-205. https://doi.org/10.1186/s12866-020-02010-3
17. John BK, Abraham T, Mathew B. A Review on Characterization Techniques for Carbon Quantum Dots and Their Applications in Agrochemical Residue Detection. J Fluoresc. 2022;32(2):449-71. https://doi.org/10.1007/s10895-021-02852-8
18. Balouiri M, Sadiki M, Ibnsouda SK. Methods for in vitro evaluating antimicrobial activity: A review. J Pharm Anal. 2016;6:71-9. https://doi.org/10.1016/j.jpha.2015.11.005
19. Pajavand H, Mobarez AM, Nikkhah M, Delnavazi MR, Abiri R. Synthesis of C-dot by hydrothermal method and evaluation of its anti-bacterial effect against antibiotic resistant S . areus and K. pneumonea. Nanomed Res. 2023;8(2):167-76. https://doi.org/10.22034/nmrj.2023.02.006
20. Sadaqat MH, Mobarez AM, Nikkhah M. Curcumin carbon dots inhibit biofilm formation and expression of esp and gelE genes of Enterococcus faecium. Microb Pathog. 2022;173(Mic):9-10. https://doi.org/10.1016/j.micpath.2022.105860
21. Mura S, Ludmerczki R, Stagi L, Garroni S, Carbonaro CM, Ricci PC, et al. Integrating sol-gel and carbon dots chemistry for the fabrication of fluorescent hybrid organic-inorganic films. Sci Rep. 2020;10(1):1-13. https://doi.org/10.1038/s41598-020-61517-x
22. Tadesse A, Hagos M, Ramadevi D, Basavaiah K, Belachew N. Fluorescent-Nitrogen-Doped Carbon Quantum Dots Derived from Citrus Lemon Juice: Green Synthesis, Mercury(II) Ion Sensing, and Live Cell Imaging. ACS Omega. 2020;5(8):3889-98. https://doi.org/10.1021/acsomega.9b03175
23. Wang M, Liu M, Nong S, Song W, Zhang X, Shen S, et al. Highly Luminescent Nucleoside-Based N, P-Doped Carbon Dots for Sensitive Detection of Ions and Bioimaging. Front Chem. 2022;10(June):1-11. https://doi.org/10.3389/fchem.2022.906806
24. Sadeghi Dousari A, Satarzadeh N. The Spread of Carbapenemase Genes in Klebsiellapneumoniae in Iran: a Systematic Review. Int J Basic Sci Med. 2021;6(1):1-10. https://doi.org/10.34172/ijbsm.2021.01
25. Abdelhalim MM, Saafan GS, El-Sayed HS, Ghaith DM. In vitro antibacterial effect of probiotics against Carbapenamase-producing multidrug-resistant Klebsiella pneumoniae clinical isolates, Cairo, Egypt. J Egypt Public Health Assoc. 2022;97(19). https://doi.org/10.1186/s42506-022-00114-4
26. 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):106821. https://doi.org/10.1016/j.jece.2021.106821
27. Seil JT, Webster TJ. Antimicrobial applications of nanotechnology: Methods and literature. Int J Nanomedicine. 2012;7:2767-81. https://doi.org/10.2147/IJN.S24805
28. Knoblauch R, Geddes CD. Carbon nanodots in photodynamic antimicrobial therapy: A review. Materials (Basel). 2021;6:1-10. https://doi.org/10.34172/ijbsm.2021.01
29. Wu Y, Li C, van der Mei HC, Busscher HJ, Ren Y. Carbon quantum dots derived from different carbon sources for antibacterial applications. Antibiotics. 2021;10(6):623. https://doi.org/10.3390/antibiotics10060623
30. Abdullah NA, Mahmoud HE, El-Nikhely NA, Hussein AA, El-Khordagui LK. Carbon dots labeled Lactiplantibacillus plantarum: a fluorescent multifunctional biocarrier for anticancer drug delivery. Front Bioeng Biotechnol. 2023;11(May):1-17. https://doi.org/10.3389/fbioe.2023.1166094
31. Hallaji Z, Bagheri Z, Oroujlo M, Nemati M, Tavassoli Z, Ranjbar B. An insight into the potentials of carbon dots for

in vitro live-cell imaging: recent progress, challenges, and prospects. Microchim Acta. 2022;189(5):35419708. https://doi.org/10.1007/s00604-022-05259-9

32. Phan LMT, Cho S. Fluorescent Carbon Dot-Supported Imaging-Based Biomedicine: A Comprehensive Review. Bioinorg Chem Appl. 2022;2022. https://doi.org/10.1155/2022/9303703
33. Fu T, Wan Y, Jin F, Liu B, Wang J, Yin X, et al. Efficient imaging based on P - and N-codoped carbon dots for tracking division and viability assessment of lactic acid bacteria. Colloids Surfaces B Biointerfaces. 2023;223:1-2. https://doi.org/10.1016/j.colsurfb.2023.113155
34. Boakye-Yiadom KO, Kesse S, Opoku-Damoah Y, Filli MS, Aquib M, Joelle MMB, et al. Carbon dots: Applications in bioimaging and theranostics. Int J Pharm. 2019;564:308-17. https://doi.org/10.1016/j.ijpharm.2019.04.055
35. Molaei MJ. Carbon quantum dots and their biomedical and therapeutic applications: A review. RSC Adv. 2019;9(12):6460-81. https://doi.org/10.1039/C8RA08088G
36. Pajavand H, Mobarez AM, Barati A, Nikkhah M, Delnavazi MR, Abiri R, et al. Evaluation of combined carbon dots and ciprofloxacin on the expression level of pslA, pelA, and ppyR genes and biofilm production in ciprofloxacin-resistant Pseudomonas aeruginosa isolates from burn wound infection in Iran. J Glob Antimicrob Resist. 2023;35:289-96. https://doi.org/10.1016/j.jgar.2023.10.005