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Research article

Synergistic effect of Polyhydroxybutyrate nanoparticle on the susceptibility of *Escherichia coli* to cefotaxime *in vitro*

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ABSTRACT

Multidrug-resistant uropathogenic *Escherichia coli* poses a major therapeutic challenge. Nanotechnology is an alternative strategy to improve the effectiveness of conventional antibiotics. The synergistic effect of nanoparticles in restoring antibiotic susceptibility is scarce in the literature. The study aims to evaluate the synergistic effect of polyhydroxybutyrate (PHB) nanoparticles on UPEC susceptibility to cefotaxime (CTX) using a checkerboard assay. The effect of the combination on UPEC's ability to form biofilms was evaluated. The UPEC was isolated from 83 urine samples. The most isolates were resistant to CTX (5/10), and the minimum inhibitory concentration (MIC) ranged from 0.062 to 256 µg/mL. Biofilm formation showed a significant correlation with resistance to CTX. The PHB NPs were synthesized and characterized by scanning electron microscopy, which revealed a diameter of 10-75 nm, and by FTIR spectroscopy, which confirmed polymer integrity. The cytotoxicity assessment against MCR-7 cells yielded an IC50 of 52.4 µg/mL. Checkboard microdilution assays against the resistant and strong biofilm-form isolates of *E. coli* (Ec9) showed that PHB NPs enhanced CTX activity, decreasing its MIC from 250 µg/mL to 62.5 µg/m. The combination of ¼ MIC PHB NPs produced the lowest fractional inhibitory concentration (FICI; 0.50), indicating a synergistic interaction. The sub-MIC combination of the two agents significantly reduced biofilm formation compared to either agent alone, resulting in an optical density (OD) decrease from 0.83 to 0.13. From the current study, it can be concluded that PHB NPs represent a promising adjunctive strategy for restoring antibiotic activity. These results need further research to achieve the final goal of reactivating conventional antibiotics.

Keywords: Antimicrobial resistance; Biofilm eradication; Biopolymer nanoparticles; Cefotaxime; Drug-resistant; Green nanotechnology; Nanomedicine; PHP-NPs; Uropathogenic E. *coli*.

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1. INTRODUCTION

The resistance to antibiotics is considered a global challenge that faces public health, responsible for increasing the rate of morbidity and mortality and healthcare costs worldwide [1]. It has has been reported previously that *Escherichia coli* isolates cause various infections, including urinary tract infections (UTIs), bacteremia, wound infections, and neonatal sepsis [2].

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Recently, *E. coli* has increasingly acquired resistance to different kinds of antibiotics, almost through the production of extended-spectrum β-lactamases (ESBLs), efflux pump overexpression, and porin alterations, which restrict antibiotics' penetration and enter the bacterial cells [3].

Cefotaxime (CTX) is a 3rd-generation cephalosporin. It has been considered for many years a drug of choice for infections caused by various pathogenic bacteria because of its broad-spectrum activity and relative safety. In contrast, the rising outbreak of ESBL-producing *E. coli* has substantially compromised its clinical effectiveness, necessitating the development of new strategies to reactivate and restore this antibiotic susceptibility [4].

Recently, nanotechnology has provided promising avenues to struggle antibiotic resistance by enhancing the antibiotic delivery, increasing antibiotic potency, and improving synergistic interactions between nanoparticles and conventional antibiotics [5]. The nanoparticles are considered an adjuvant capable of changing bacterial membrane permeability and enhancing antibiotic action [6].

Polyhydroxybutyrate (PHB) is a naturally occurring biopolyester synthesized by different bacteria and plants. It has significant interest not only for this material's biodegradability and biocompatibility, but also for its antimicrobial properties, especially antibacterial effects, and its ability to interact with the cell membrane when PHB is formulated into nanoparticles [7]. Several studies have reported the biomedical applications of PHB, but limited data are available on their interactions with conventional antibiotics and their potential role in reducing pathogenic bacterial resistance to antibiotics [7, 8].

No previous study has demonstrated the synergistic effect of PHB-NPs on the effectiveness of cefotaxime against *E. coli* isolated from wound infections. This project opens the door to new, safe strategies for restoring the efficacy of conventional antibiotics (cefotaxime) against multidrug-resistant *E. coli* isolated from infected wounds. Thus, the present study aims to assess the synergistic effect of PHB-NPs on the susceptibility of MDR *E. coli* to CTX.

2. MATERIALS AND METHODS

2.1. Bacterial Isolates

In the current study, 83 urine samples were collected from the same number of patients. The patients attended two main hospitals in Baghdad, Iraq. The patients stoped to take antibiotic therapy within 72 h prior to sample collection, and informed consent was obtained from all participants. The samples were inoculated onto various differential and selective culture media, including MacConkey agar. Pink colonies (lactose-fermented colonies) were selected. Gram stain was performed, followed by biochemical tests. A VITEK DensiCheck instrument and fluorescence system (bio-Mérieux, Marcy-l'Étoile, France) (ID-GNB card) were used to finally identify the isolates as *E. coli*.

2.2. Kirby-Bauer disc diffusion method

The Kirby-Bauer disk diffusion method described previously [9,10] was used to determine the antibiotic susceptibility of cefotaxime (CTX) against 10 *E. coli* isolates. Inhibition zone diameters, including the 6 mm disk diameter, were measured in millimeters. The Clinical and Laboratory Standards Institute (CLSI) M100 guidelines breakpoints for CTX were used to interpret the results and categorize them as susceptible (S, sensitive to CTX), intermediate (I), or resistant (R).

2.3. PHB-NPs preparation and characterization

The PHB-NPs were prepared using a precipitation-solvent evaporation emulsification assay. Hundred milligrams of PHB polymer (Sigma-Aldrich, USA) were dissolved in ten milliliters of chloroform (Fluke, UK) with mixing at 20 °C. The organic polymer solution was added drop by drop to 100 mL of 0.5% polyvinyl alcohol (Sigma-Aldrich, USA) with ultrasonication (Sonics Vibra-cell, USA) at 40% amplitude for 5 min in an ice bath. The resulting emulsion was incubated overnight with gentle mixing at 20 °C, producing stable aqueous PHB-NPs. The yielded PHB-NPs were harvested by centrifugation at 10,000xg for 20 min. The yielded production was washed three times with double-distilled water. The final product was stored at -20 °C until use. Morphological characterization was performed using scanning electron microscopy (SEM; Hitachi High-Tech, Tokyo, Japan) after negative staining with 2% uranyl acetate. Fourier-transform infrared (FTIR) spectroscopy (Perkin-Elmer Spectrum 65; PerkinElmer, USA) was used.

2.4. MTT Assay

In the current experiment, the cytotoxicity of PHB-NPs on MCF-7 human breast cancer cells was evaluated using the MTT assay, a colorimetric indicator of cell viability. The method was described in detail in previous studies [11-13]. Cell viability was expressed as a percentage relative to untreated control cells using the following formula:

$$\text{Cell viability (\%)} = \left(\frac{\text{Abs}_{\text{treated}}}{\text{Abs}_{\text{control}}}\right) \times 100$$

The IC50 was determined from the concentration-response curve.

2.5. Minimum inhibitory concentrations (MICs)

The MICs of CTX and PHB-NPs were determined by the microdilution method in 96-well U-shaped polystyrene microtiter plates (Thermo Scientific, USA) following standard procedures [9,10]. Serial double-fold dilutions of both agents (100 µL) were prepared in Muller-Hinton broth (MHB-HiMedia, India). The standard inoculum of *E. coli* was prepared by washing the overnight bacterial growth three times with phosphate-buffered saline (PBS, 151.5 mM, pH 7.2), then adjusting the optical density of the suspension to 0.1 at 600 nm. Five microliter of bacterial suspension was added to each well. The plates were incubated at 37 °C for 24 h. Three controls were applied. First control, growth control (MHB+bacteria); second control, sterile control (MHB only), and third control (antibiotic turbidity control). The experiments were repeated three times [9,10].

2.6. Biofilm formation

Biofilm formation in the *E. coli* isolates was measured using the crystal violet microtiter plate assay. This method was described in detail in several previous standard publications [9,10]. In this method, tryptic soy broth (TSB, Hi-Media, India) supplemented with 0.25% glucose was used. Absorbance was measured at 590 nm (Bio-Rad, USA). The absorbance of an empty well was used as the blank. Sterility of the media and the effectiveness of bacterial growth controls were verified. The experiment was repeated three times. The cut-off value was calculated to define the ranges for the three categories of bacteria that produced biofilm (strong, moderate, and weak biofilm producer isolates).

2.7. Synergistic effect of PHB-NPs and CTX on MICs

The checkerboard microdilution method was used to evaluate the effectiveness of the interaction between PHB-NPs and CTX

against the MDR *E. coli* isolate that produced a strong biofilm on polystyrene microtiter plates. In the U-shaped polystyrene microtiter plate, twofold serial dilutions of CTX were prepared from 2000 µg/mL to 0.97 µg/mL across columns 1-12. Twofold serial sub-MICs of PHB-NPs were prepared from 1/2 to 1/64 of the MIC. The agents were prepared in MHB (HiMedia, India). Five microliters of the standard inoculum of *E. coli* (preparation was described in 2.6 MICs) was added to each well and incubated at 37 ºC for 24 h. The fractional inhibitory concentration index (FICI) was calculated as: FICI = (MIC of CTX in combination / MIC of CTX alone) + (concentration of PHB NPs used / MIC of PHB NPs alone). FICI <=0.5 was defined as synergistic, 0.5-1.0 as additive, 1.0-2.0 as indifferent, and >2.0 as antagonistic effect.

2.8. Effect of the Combination of CTX and PHB-NPs at sub-MICs on Biofilm

The procedure of evaluation of the synergistic effect of studied agents on *E. coli* (2.8) was followed to check the effect of the combination of CTX and PHB-NPs at sub-MIC levels on the ability of the *E. coli* isolate to form biofilm on a flat-bottom polystyrene microtiter plate. In the experiment, the TSB (HiMedia, India) was used instead of MHB. After incubation, the biofilm measurement protocol was followed. The absorbance was measured at 590 nm using a microplate reader (Bio-Rad, USA). The experiment was repeated three times [14-16].

2.9. Statistical analysis

The Microsoft excel and IBM SPSSv.26 (IBM, USA) were used in the analysis of the current study's data. The data were expressed in mean ± SD. The student's t-test and one-way ANOVA with a post hoc Tukey's test were used for comparison. A p-value of <0.05 was considered statistically significant.

3. RESULTS

3.1. Bacterial isolates and CTX susceptibility

In the present study, 10 *E. coli* isolates were obtained from 83 urine samples collected from patients with UTIs. The incidence of the UTI infection with *E. coli* was 12.04. The species were identified using the VITEK® 2 system. The Kirby–Bauer disk diffusion method showed that the inhibitory zone diameters of the 10 *E. coli* isolates ranged from 7.8 ± 0.9 mm (Ec9) to 32.1 ± 3.5 mm (Ec8). All reported inhibition zone diameters represent the total diameter, including the 6 mm disk diameter, consistent with CLSI measurement guidelines. The MIC values of CTX were ranged from 0.062 µg/mL to 256 µg/mL. Of the 10 isolates, 4 isolates were susceptible (S), 1 intermediate (I), and 5 resistant (R) to CTX, indicating a high prevalence of CTX resistance in uropathogenic *E. coli* (Table 1). The microdilution method was used to determine the MICs of PHB-NPs against 10 *E. coli* isolates. The range of MICs was from 0.12 µg/mL to 256 µg/mL.

3.2. Biofilm formation and CTX response

The study showed that six isolates were categorized as strong biofilm producers, three as moderate, and one as a weak biofilm producer. It was observed that the susceptible isolates showed low to moderate biofilm production, while resistant isolates exhibited moderate to strong biofilm formation. The statistical analysis showed a negative correlation between biofilm formation and inhibition zone diameter (r = -0.83; p = 0.007). The study also showed a significant positive correlation between biofilm formation and CTX MICs (r = +0.801, p = 0.003). These data support that enhanced biofilm production is strongly associated with increased CTX resistance (Table 1).

Table 1. The susceptibility of CTX of ten isolates of *E. coli* and their capacity to produce biofilm. Inhibitory zone diameter was measured in millimeters (mm), minimum inhibitory concentrations (MICs) were measured in µg/mL, and biofilm formation was measured in OD at 590 nm. The ten isolates were classified as susceptible (S), intermediate (I), or resistant (R) to CTX based on CLSI breakpoints. The last column exhibits the effect of PHB-NPs at ½ MICs on biofilm formation (OD 590 nm).

Isolate	Inhibition Zone (mm) CTX	MIC (µg/mL) CTX	Category	Biofilm (OD 590 nm)	PHB NPs MIC (µg/mL)
Ec1	28.3 ± 3.1	0.06	S	0.22 ± 0.09	0.12
Ec2	11.2 ± 1.8	64	R	0.62 ± 0.17	256
Ec3	22.5 ± 2.7	2	I	0.44 ± 0.12	8
Ec4	8.6 ± 1.3	256	R	0.79 ± 0.22	128
Ec5	25.1 ± 3.0	0.5	S	0.34 ± 0.11	1
Ec6	29.4 ± 4.1	0.06	S	0.30 ± 0.10	8
Ec7	15.3 ± 2.0	32	R	0.57 ± 0.20	64
Ec8	32.1 ± 3.5	0.06	S	0.20 ± 0.14	0.24
Ec9	7.8 ± 0.9	256	R	0.81 ± 0.25	256
Ec10	13.0 ± 1.5	128	R	0.70 ± 0.19	128

3.3. PHB-NPs characterization

Scanning electron microscopy revealed that the PHB nanoparticles exhibited a near-spherical morphology with aggregation. The FTIR spectra showed the characteristic PHB carbonyl ester peak at 1700 cm-1 and C-O-C stretching bands at 1278 and 1060 cm-1. These findings confirmed the identity of the prepared PHB nanoparticles and the integrity of the polymer core.

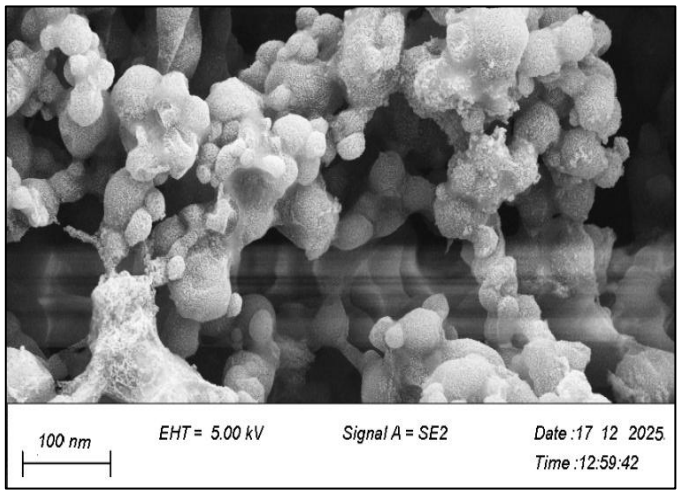

Fig. 1. Scanning electron microscopy (SEM) of PHB-NPs exhibiting aggregated nanoscale particles with semi-spherical shape and rough surface texture. The diameter of the PHB-NPs in the image ranged from 10 to 75 nm.

3.4. MTT Cytotoxicity Assay

The results showed that PHB NPs exhibited a concentration-dependent decrease in MCF-7 cell viability. The inhibition concentration. The half-maximal inhibitory concentration (IC50) was determined to be 52.4 ug/mL (Fig. 2). The sub-MIC concentrations of PHB NPs used in the synergistic assay were below the IC50 value. That refers to a potential therapeutic selectivity window, indicating the feasibility of the nanoparticle concentrations used in the *in vitro* antibacterial assays.

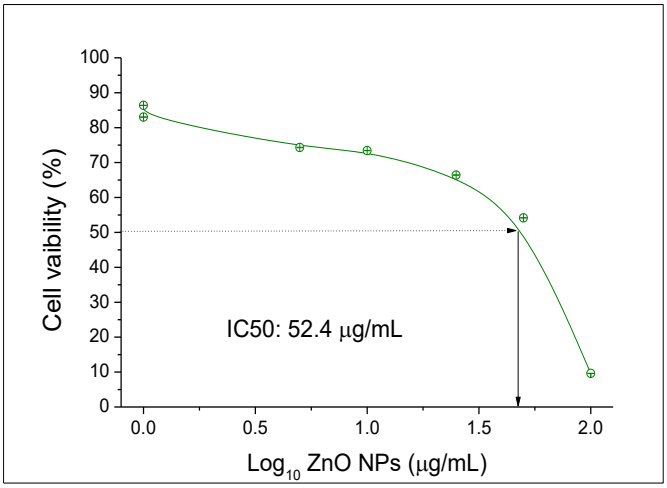

Fig 2. Cytotoxic effect of PHB NPs against MCF-7 cells assessed by MTT assay. Concentration response curve showing percentage cell viability plotted against the $\log_{10}$ concentration of PHB NPs. The half-maximal inhibitory concentration (IC_{50}) was 52.4 µg/mL.

3.5. Effect of the Combination of PHB NPs and CTX on Biofilm

Table 4 shows the effect of combining PHB NPs and CTX at sub-MIC levels on biofilm formation by Ec9. The lowest biofilm formation, measured by OD590, was observed in the untreated control (0.83 ± 0.21) and decreased to 0.13 ± 0.04 (P<0.05 vs. the first, second, and third controls) at the highest combination concentrations (1/2 MIC PHB NPs and ½ MIC CTX). At the lowest concentrations of the combined agents (1/32 MIC PHB NPs and 1/32 MIC CTX), biofilm production was significantly reduced compared with all controls (P<0.05). Thus, the current experiments report, for the first time, that the combination of CTX and PHB NPs at sub-MIC levels reduced *E. coli* biofilm formation more effectively than either agent applied alone.

Tables 2 and 3 demonstrated that the combination of PHB NPs and CTX increase the antibacterial activity of CTX against *E. coli* (Ec9). The MIC of CTX was 250 µg/mL (control only CTX); however, when combined with PHB NPs, bacterial growth inhibition was seen at a lower CTX concentration of 62.5 µg/mL when the ½ and ¼ MICs of PHB NPs were used. The MICs of CTX were reduced to 125 µg/mL when combined with 1/8, 1/16, and 1/32 of CTX sub-MICs. The combinations exhibited synergistic interactions based on the FICI values. The strongest synergistic effect was observed with ¼ MIC PHB NPs combined with CTX, which produced the lowest FICI value (0.5). In contrast, the combination containing 1/2 MIC PHB NPs showed an additive effect (FICI = 0.75).

Table 3. Fractional inhibitory concentration index (FICI) values of the PHB NPs and CTX combination against EC9. ≤ 0.5, synergistic effect; 0.5-1.0, additive effect; 1.0-2.0, indifferent, ≥ 2.0, antagonistic effect.

PHB-NPs	FIC PHB	FIC CTX	FICI	Interpretation
½ MIC	0.50	62.5/250 = 0.25	**0.75**	Additive/Partial synergy
¼ MIC	0.25	0.25	**0.50**	**Synergistic**
⅛ MIC	0.125	125/250 = 0.50	**0.625**	Additive
1/16 MIC	0.0625	0.50	**0.5625**	Additive
1/32 MIC	0.0313	0.50	**0.5313**	Additive
1/64 MIC	0.0156	0.50	**0.5156**	Additive

3.6. Effect of the Combination of PHB NPs and CTX on Biofilm

Table 4 shows the effect of combining PHB NPs and CTX at sub-MIC levels on biofilm formation by Ec9. The lowest biofilm formation, measured by OD590, was observed in the untreated control (0.83 ± 0.21) and decreased to 0.13 ± 0.04 (P<0.05 vs. the first, second, and third controls) at the highest combination concentrations (1/2 MIC PHB NPs and ½ MIC CTX). At the lowest concentrations of the combined agents (1/32 MIC PHB NPs and 1/32 MIC CTX), biofilm production was significantly reduced compared with all controls (P<0.05).

Table 2. Checkerboard microdilution method exhibiting the synergistic antibacterial interaction between PHB NPs and CTX against *E. coli* (EC9) isolate. The symbol (−) indicates no visible bacterial growth (antibacterial effect), whereas (+) indicates visible bacterial growth. Different sub-MICs of PHB NPs were combined with serial sub-MICs of CTX.

	CTX µg/mL											Control Only PHB NP
	1000	500	250	125	62.5	31.25	15.62	7.8	3.9	1.9	0.97	
MIC PHB NP	-	-	-	-	-	-	-	-	-	-	-	-
½ MIC PHB NP	-	-	-	-	-	+	+	+	+	+	+	+
¼ MIC PHB NP	-	-	-	-	-	+	+	+	+	+	+	+
1/8 MIC PHB NP	-	-	-	-	+	+	+	+	+	+	+	+
1/16 MIC PHB NP	-	-	-	-	+	+	+	+	+	+	+	+
1/32 MIC PHB NP	-	-	-	-	+	+	+	+	+	+	+	+
1/64 MIC PHB NP	-	-	-	-	+	+	+	+	+	+	+	+
Control only CTX	-	-	-	+	+	+	+	+	+	+	+	+

Thus, the current experiments report, for the first time, that the combination of CTX and PHB NPs at sub-MIC levels reduced *E. coli* biofilm formation more effectively than either agent applied alone.

4. DISCUSSION

The elevation of resistance in *E. coli* to third-generation cephalosporins represents one of the biggest challenges in infectious disease medicine and public health in general. The World Health Organization's 2024 priority pathogens list identifies ESBL-producing *E. coli* as a high-priority target for developing new treatments for infectious diseases [17]. The present study showed that a high percentage of uropathogenic *E. coli* isolates were resistant to cefotaxime (CTX), confirming the high outbreak of ESBL-producing *E. coli* documented from Iraqi clinical settings and reflecting global trends in beta-lactam resistance [18].

The study showed a negative correlation between the biofilm formation of *E.* coli isolates and the CTX inhibition zone diameter, and a positive correlation was observed between the minimum inhibitory concentration of the same isolates and their biofilm formation. Thus, the biofilm biomass restricts the antibiotic diffusion, reduces the pH and oxygen tension, helping in the bacteria persistence, and that develops the resistance gene expression, which explains the correlation between the biofilm formation and the resistance to antibiotics, especially to CTX [19, 20].

The prepared PHB NPs exhibited physicochemical characteristics, including nano-diameter, that are well suited for biomedical applications. The nanoscale size of the PHB NPs facilitates their interaction with bacterial cell surfaces and biofilm matrix components, enhancing penetration into the biofilm matrix. The study also showed that the IC50 of the prepared PHB NPs against MCF-7 cells was 52.4 µg/ml, supporting the concept of using this material in therapeutic fields. Several previous studies have highlighted the use of PHB material at various sizes, including nanoscale, in therapeutic and antimicrobial applications [21].

There are several mechanisms that underlie potentiation of the action of CTX by PHB NPs. The physical characteristics of PHB NPs (i.e., nanoscale size and hydrophobicity of the NPs) play a role in facilitating the adhesion of the PHB NPs to the bacterial outer membrane and disrupting the packing of LPS, which would increase the permeability of the membrane to CTX, thus allowing CTX better access to its PBP targets. PHB-based NPs may also interfere with efflux pump function by changing the fluidity of the membrane or altering the conformation of membrane proteins, decreasing the amount of CTX that is extruded from inside the cell by the efflux pump [22]. Within biofilms, PHB NPs can penetrate and interact with the polysaccharides and proteins in EPS, thereby disrupting the structural integrity of the biofilm and providing a lower resistance to the diffusion of CTX. These processes are consistent with the known antibiofilm activity of NP systems based on other polymers against Gram-negative pathogens [23,24].

The outcomes of this study support that PHB nanoparticles display an additive or synergistic capacity with ceftriaxone (CTX) against *E. coli* using combined FIC index values; these outcomes correlate with previous studies evaluating the additive interactions between polymers and antibiotics [25]. In addition to this, and although at the maximum concentration tested (256 g/mL 120.8 g/mL) there were significant reductions in the MICs of CTX against Ec9 (compared to baseline MIC (256 µg/mL), Ec9 remained clinically resistant to CTX based on MIC testing, as all of the post-combination MIC values were still well above the clinical susceptibility breakpoint of 1 µg/mL as established by the Clinical Laboratory Standards Institute (CLSI). Thus, the authors do not claim that the PHB NPs restored clinical susceptibility to CTX, but rather that the NPs potentiated the CTX's actions. However, the data collected from FIC studies support the need for more studies involving extended pharmacokinetic/pharmacodynamic modeling and *in vivo* validation before clinical translation can take place.

Combined PHB NPs and CTX tested displayed a greater antibiofilm efficacy than either agent alone, based on the amount of biofilm EP (extracellular polymeric substance) biomass produced from Ec9, with up to an 85% reduction relative to the untreated control for the maximum concentration tested. This finding is in agreement with previous studies that showed the combined activities of polymer NPs and antibiotics are greater than those of single antibiotics when penetrating/disrupting biofilms [26].

The current study has many limitations. 1st, the study focuses on the *in vitro* environment. Thus, the future studies will deal with using the mice UTI model to evaluate combined PHB NPs and CTX treatment *in vivo*. 2nd, the genetic basis of resistance determination was not investigated and will be addressed in subsequent investigations. 3rd, the synergistic effect of both agents was tested on one isolate (Ec9), and a similar approach was used to assess the effect of the combination of both agents on the biofilm formation of one isolate (Ec9). Thus, the future study will address this point carefully by employing several isolates in the study.

Table 4 The biofilm formation of *E. coli* (Ec9) (OD590 nm) post incubation at 37 °C for 24 h in exposure to PHB NPs, CTX, and their combination at sub-MIC levels. 1st control: biofilm formation of the bacteria under CTX cub-MICs alone; 2nd control: biofilm formation of the same bacteria under sub-MICs of PHB NPs alone; 3rd control: the biofilm formation of the untreated bacteria. # p < *0.05* vs 1st control; ^ *p<0.05* vs second control; *p< 0.05* vs 3rd control.

Sub-MICs PHB NPs	1/2 MIC CTX	1/4 MIC CTX	1/8 MIC CTX	1/16 MIC CTX	1/32 MIC CTX	2nd Control
1/2 MIC PHB NPs	0.13 ± 0.04*#^	0.16 ± 0.04*#^	0.18 ± 0.04*#^	0.19 ± 0.04*#^	0.21 ± 0.05*#^	0.31 ± 0.04
1/4 MIC PHB NPs	0.15 ± 0.04*#^	0.18 ± 0.05*#^	0.19 ± 0.04*#^	0.22 ± 0.05*#^	0.25 ± 0.06*#^	0.34 ± 0.05
1/8 MIC PHB NPs	0.18 ± 0.05*#^	0.17 ± 0.04*#^	0.20 ± 0.05*#^	0.23 ± 0.06*#^	0.30 ± 0.06*#^	0.51 ± 0.04
1/16 MIC PHB NPs	0.16 ± 0.05*#^	0.20 ± 0.06*#^	0.19 ± 0.06*#^	0.24 ± 0.06*#^	0.33 ± 0.07*#^	0.56 ± 0.03
1/32 MIC PHB NPs	0.17 ± 0.04 *#^	0.21 ± 0.07*#^	0.22 ± 0.07 *#^	0.23 ± 0.08*#^	0.34 ± 0.09*#^	0.66 ± 0.23
1st control	0.34 ± 0.08*#^	0.43 ± 0.09*#^	0.53 ± 0.12*#^	0.59 ± 0.13*#^	0.62 ± 0.18*#^	3rd control (0.083 0.21

5. CONCLUSION

The present study's findings provide evidence that 50% of uropathogenic *E. coli* isolates are resistant to CTX and show a significant correlation between biofilm-forming ability and CTX resistance. PHB NPs created by solvent evaporation with emulsification have shown suitable physicochemical characteristics and exhibit low toxicity (IC50: 52.4 ug/mL). Sub-MICs of CTX and PHB NPs alone have shown a concentration-dependent reduction in biofilm formation. This study is the first to show that PHB NPs have a combined effect with CTX on both killing CTX-resistant *E. coli* and inhibiting biofilm formation, supporting *in vivo* testing to further evaluate their use in conjunction with CTX as adjunctive antimicrobial therapy for treating drug-resistant *E. coli* and preventing biofilm-related persistence.

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Conflict of interest

The authors declare no conflicts of interest.

Ethical Approval

This review was approved by the Ministry of Health, Baghdad, Iraq (1104; 16-04-2025).

Author contributions

Aggarw K. Conceptualization, Methodology, Investigation, Formal analysis, Writing – original draft. Writing – review & editing.

AL-Saadi AH: Conceptualization, Investigation, Supervision, Methodology, Validation, Writing – review & editing.

Heydarl MM: Data curation, Formal analysis, Visualization, Writing – review & editing.

Sharma M. Investigation, Resources, Validation, Data curation, Writing – review & editing.

Alshahran SM. Supervision, Project administration, Resources, Funding acquisition, Writing – review & editing.

All authors reviewed and approved the final manuscript and agreed to be accountable for all aspects of the work.

Data availability

Data will be made available on request.

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