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REVIEW ARTICLE

Efflux Pump and Biofilm Inhibitory Activity of Nanoparticles in *Acinetobacter Baumannii*: a Systematic Review

Pegah Shakib [1], Reza Saki [2], Mohammad R. Zolfaghari [3], Gholamreza Goudarzi [1]

[1] *Razi Herbal Medicines Research Center, Lorestan University of Medical Sciences, Khorramabad, Iran*
[2] *Department of Microbiology, Kermanshah University of Medical Sciences, Kermanshah, Iran*
[3] *Department of Microbiology, Qom Branch, Islamic Azad University, Qom, Iran*

SUMMARY

Background: ***Acinetobacter baumannii*** **produce biofilm and efflux pumps. This systematic review study aimed to provide new strategies to inhibit the efflux pumps and biofilm in *A. baumannii* using nanoparticles.**
Methods: **In this research, analyses from 2000 to February 24, 2022, were performed by the Statement of Preferred Reporting Items for Systematic Reviews (PRISMA). Keywords include *Acinetobacter baumannii* (*A. baumannii*) AND (biofilm) AND (anti-biofilm activity) AND (nanoparticles) AND (solid lipid NPS) AND (lipid nanocarriers), and in other searches include *Acinetobacter baumannii* (*A. baumanni*) AND (efflux pumps) AND (nanoparticles) AND (solid lipid NPS) AND (lipid nanocarriers). Searches were conducted in English databases, including Science Direct, PubMed, Scopus, Ovid, and Cochrane.**
Results: **At first, 136 studies were extracted, but after removing duplicates, 116 cases remained for further analysis. After evaluating the title and abstract of each study, 95 unrelated studies were excluded. The remaining 25 studies were reviewed based on full texts. Considering the inclusion/exclusion criteria, 19 studies were selected. In this study, metal nanoparticles were the most used nanoparticles for anti-biofilm and efflux pump purposes, and among these nanoparticles, silver nanoparticles (AgNPs) contributed the most.**
Conclusions: **The present study shows that nanoparticles have potential and significant effects in inhibiting biofilm and efflux pumps in *A. baumannii* isolates, which researchers can consider in light of the increasing prevalence of antibiotic resistance.**
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Correspondence:
Gholamreza Goudarzi
School of Medicine
Kamalvand Campus
Lorestan University of
Medical Sciences
Khorramabad
Iran
Phone: +98 6633204005
E-mail: Goudarzi.gh@gmail.com

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KEYWORDS

anti-biofilm, *Acinetobacter baumannii*, efflux pump, systematic review, nanoparticle

INTRODUCTION

Acinetobacter baumannii (*A. baumannii*) is a non fermentative, gram-negative coccobacillus abundant in soil, water, and hospital environment. *A. baumannii* is an opportunistic pathogen that causes nosocomial infections such as bacteremia, pneumonia, meningitis, and urinary tract infections [1,2]. Antibiotic treatment of these infections often involves broad-spectrum beta-lactams, aminoglycosides, and fluoroquinolones. But today, we are witnessing the spread of multidrug-resistant phenotypes (MDR) among the clinical strains of *A. bau-*

mannii from around the world [3,4]. Increased antibiotic resistance leads to limited treatment options, longer treatment periods, increased costs, and treatment failure, thus posing a serious threat to human health. Biofilm formation is one of the most effective factors in creating drug resistance [5,6]. Forming biofilm on the mucosa, medical devices, and equipment can increase antibiotic resistance and resistance to other factors, including resistance to the host immune response, phagocytic factors, and environmental stress [7]. A biofilm is a complex accumulation of microbial colonies that forms an acellular matrix composed of a protective polysaccharide layer (exopolysaccharide; EPS) [8]. According to the US National Institutes of Health, biofilms are responsible for 80% of infections in the body [9]. Numerous factors, including outer membrane proteins A (OmpA) and Biofilm-Associated Protein (BAP), are involved in biofilm formation [10]. BAP is a high molecular weight protein at the bacterial level that contains a central nucleus consisting of successive repeats of similar sequences [11]. Another mechanism of antibiotic resistance in *A. baumannii* is the overexpression of efflux pumps. Nowadays, the role of efflux pumps in antibiotic resistance has attracted the attention of researchers. AdeABC, AdeIJK, and eFGH can be mentioned among the efflux pumps in *A. baumannii* [12,13].

Due to the spread of antibiotic resistance in clinical isolates of biofilm-producing *A. baumannii* and expression efflux pumps, researchers have begun to use nanoparticles to treat these infections to find new therapies [13, 14]. The nanoparticles attach to the bacterial membrane through an electrostatic reaction and completely disrupt the cell membrane [15]. One of the antimicrobial mechanisms of nanoparticles is the reaction with amines and carboxyl groups of the peptidoglycan layer of the bacterial wall, which causes damage to the bacterial cell wall. The synthesis of nanoparticles can be done chemically and physically. Therefore, the use of nanoparticles and their synthesis can provide a safe, new, and promising solution in the health and safety field in the control of pathogenic bacteria [16,17]. This systematic review study aimed to provide new strategies to inhibit the efflux pump and biofilm in *A. baumannii* using nanoparticles.

MATERIALS AND METHODS

We did our analyses from 2000 to Feb 24, 2022, by the Preferred Reporting Items for Systematic Reviews (PRISMA) statement.

Search strategy and selection criteria

The methodology for this systematic review is based on screening the literature online in English and Persian. Searching English databases included Science Direct, PubMed, Scopus, Ovid, and Cochrane. Specifically, we searched for *Acinetobacter baumannii* (*A. baumannii*) AND (biofilm) AND (antibiofilm activity) AND (nanoparticles) AND (solid lipid NPS) AND (nano lipid carriers).

In addition, in another search, we used the keywords *Acinetobacter baumannii* (*A. baumannii*) AND (efflux pumps) AND (expression efflux pumps) AND (nanoparticles) AND (solid lipid NPS) AND (lipid nanocarriers).

Two authors evaluated the articles independently, and discrepancies were resolved through discussion when there were disagreements.

Inclusion and exclusion criteria

Inclusion criteria

The inclusion criteria of this study are as follows:

Specifically, studies that have evaluated the effect of nanoparticles on the expression of efflux pumps and biofilm-producing *A. baumannii* from 2000 to Feb 24, 2022*;* studies published in Persian and English; studies published in peer-reviewed journals; studies with clear information to evaluate; studies for which full-text is available; studies that have evaluated the effect of nanoparticles on *A. baumannii* only.

Exclusion criteria

The following studies were excluded from this study: studies for which full text was unavailable; studies that were case reports, case series, systematic reviews, summaries of presentations at seminars and conferences; studies without clear information to evaluate.

Data Extraction

Two authors independently extracted the initial information from the selected articles, which include the following: author, year, type of nanoparticle, drug, and concentration.

RESULTS AND DISCUSSION

Selected Studies

After searching based on inclusion and exclusion criteria, including removing duplicates and full text, all collected articles were entered into Endnote X9 by the researchers. Finally, the main articles were selected. At first, 136 studies were extracted, but 116 cases remained for further analysis after removing duplicates. After evaluating the title and abstract of each study, 95 unrelated studies were excluded. The remaining 25 studies were reviewed based on full texts. Considering the inclusion/exclusion criteria, 19 studies were selected (Figure 1).

The results of the literature search and the characteristics of each study, including author (year), type of nanoparticles, drug/natural compounds, and concentration, are shown in Table 1. In this study, metal nanoparticles were the most used nanoparticles for anti-biofilm and efflux pump purposes, and among these nanoparticles, silver nanoparticles (AgNPs) contributed the most, followed by polymeric nanoparticles, zinc oxide nanoparti-

cles (ZNONPs), and gold nanoparticles.

Polymeric NPs (Pol-NPs)

Polymeric nanoparticles have special advantages, such as increasing drug stability and controlled drug release [36]. In the study by Govindan et al., the evaluation of the anti-biofilm properties of essential oils containing chitosan against the biofilm-forming *A. baumannii* was investigated. The results showed that the complete formation of biofilm was destroyed at a concentration of 150 µg/mL. Based on the results, with the color changes of the XTT assay, it was found that the metabolic cells of the biofilm lost their virulence, and the inactivation of the biofilm metabolic product was proven. Also, the internal and external parts of the loaded chitosan essential oils were completely damaged. Hence, all *in vitro* inhibition assays and morphological examinations confirmed that the biofilm-forming *A. baumannii* lost its virulence factors, and its bacterial growth was effectively eradicated [18]. Pourhajibagher et al. found that all antimicrobial photodynamic therapy ($aPDT^{SSR}$), antimicrobial sonodynamic therapy ($aSDT^{SSR}$), and antimicrobial photo-sonodynamic therapy ($aPSDT^{SSR}$) groups based on Curcumin-Nisin-based poly (L-lactic acid) nanoparticle (CurNisNp), have anti-biofilm effects against *A. baumannii* ($p < 0.05$). In addition, CurNisNp-$aPSDT^{SSR}$ had higher anti-biofilm activity than other groups ($p < 0.05$) [21].

Scutera et al. designed a study aiming to synthesize human albumin nanoparticles coated with chitosan for the delivery of Colistin (Col/ha NPs). The results showed that Col/haNPs is an inhibitor of biofilm formation, as it inhibited biofilm 4 and 60 times more than free colistin against colistin-sensitive and resistant *A. baumannii* isolates [24]. In Hosseini et al. study, there was a decrease in the *CsuE* gene in isolates producing strong biofilm treated with Curcumin Nanoparticles compared to untreated isolates. Therefore, curcumin nanoparticles decrease the expression of *CsuE* compared to untreated samples. The inactivation of *CsuE* results in the prevention of pili production and the formation of biofilms [25]. Azevedo et al. determined the antimicrobial and anti-biofilm activity of polyethyleneimine (PEI) and PEI-based nanoparticles (nanoPEI) against *A. baumannii* and reported that PEI at concentrations corresponding to MLC, 2 x MIC, MIC, and 0.5 x MIC significantly reduced biofilm metabolic activity. Also, NanoPEI at concentrations of 0.5 x MIC and MIC significantly reduced the metabolic activity of biofilm in *A. baumannii* [26].

Madhi et al. studied the evaluation of the effect of chitosan and AgNPs and their combination with antibiotics (ciprofloxacin and gentamicin) to change the expression of efflux pumps and increase the inhibitory capacity of antibiotics against clinical *A. baumannii*, and *P. earoginosa* was investigated. Real-time PCR results showed that the expression of *abeM* efflux pumps in *A. baumannii* and *mexY* efflux pumps in *P. aeruginosa* decreased after treatment with sub-inhibitory concentrations of chitosan, chitosan NP, and their combination with ciprofloxacin and gentamicin [33]. Madhi et al. in a study described the effects of Chitosan and AgNPs laden with antibiotics on MDR *P. aeruginosa* and *A. baumannii*. They explained that the expression of *mexB* in *P. aeruginosa* and efflux pump *adeB* in *A. baumannii* decreased after treatment with AgNPs, chitosan nanoparticles, and chitosan [35].

Metal nanoparticles

Metal nanoparticles such as gold, copper, and silver are used in various scientific and industrial fields. Silver has long been considered a disinfectant, but its use was limited due to its low bactericidal properties and the development of antibiotics and antibacterial chemicals [37,38]. However, with the development of AgNPs, the reuse of silver as a potent bactericidal agent has flourished [39]. Using AgNPs and produced polydopamine, a nanocomposite coating for central venous catheter (CVC) was formulated by Neethu et al. The surface of the nanocomposite was characterized by field emission scanning electron microscopy (FE-SEM), water contact angle measurement, and Raman spectroscopy. The results showed that Mycogen AgNPs have strong anti-biofilm activity on *A. baumannii* biofilms. SEM and FE-SEM of biofilms on the surface of CVC samples proved that AgNPs at the minimum bactericidal concentration could destroy biofilms' structure and lyse bacterial cells [19]. Muzammil et al. reported that aluminum oxide nanoparticles (Al2O3 NPs) prevent EPS production, adhesion, and biofilm formation by multi-drug resistant *A. baumannii*. Based on the results, the minimum inhibitory concentration (MIC) and minimum antibacterial concentration (MBC) for Al2O3 nanoparticles were between 125 and 1,000 µg/mL. NPs caused cell membrane disruption, plus biofilm inhibition was 11.64 to 70.2%, while bacterial attachment to polystyrene surfaces was reduced to 48.8 to 51.9% in the presence of NPs [20].

The results of a study conducted by Hendianiet al. showed that 29.3% of *A. baumannii* isolates were sensitive to AgNPs and could prevent the growth of produced biofilms. Also, the results showed that synergy between AgNPs and imipenem could inhibit biofilm [22]. After synthesizing AgNPs with Eucalyptus Critriodora leaf extract and investigating its antimicrobial and anti-biofilm properties against *A. baumannii* isolates, Wintachai et al. introduced this nanoparticle as an anti-biofilm agent with an effective concentration of 0.028 µg/mL and 50% inhibition of *A. baumannii* isolates [23]. Barabadi et al. investigated the antibiofilm properties of silver nanoparticles derived from Penicillium chrysogenum (AgNPs) against *A. baumannii* strains compared to the antibiotic tetracycline and reported that AgNPs inhibited 90% of the biofilm at a concentration of 2 µg/mL, while tetracycline inhibited at a concentration of 490 µg/mL [27]. Hetta et al. determined the Antibiofilm potential of AgNPs against multidrug-resistant (MDR) *A. baumannii* and described that AgNPs inhibit-

Table1. Publications on the efficacy and activity of nanoparticles used against *A. baumannii.*

Author	Year	Type of nanoparticles	Drug/natural compounds	Concentration	Ref
Govindan	2022	Chitosan	Morinda citrifolia	150 mg/mL	[18]
Neethu	2020	AgNPs *	Algicolous endophytic fungus Penicillium polonicum	31.2 µg/mL	[19]
Muzammil	2020	Aluminium oxide (Al2O3 NPs)	-	Biofilm inhibition was 11.64 to 70.2%	[20]
Pourhajibagher	2022	Curcumin-Nisin-based poly	L-lactic acid	-	[21]
Hendiani	2015	AgNPs *	Bismuth ethandithiol-Bismuth propanedithiol imipenem	(29.3%) showed susceptibility to 100 ppm	[22]
Wintachai	2019	AgNPs *	Eucalyptus critriodora leaf	0.72 µg/mL	[23]
Scutera	2021	Human Albumin	Colistin	20 and 40 µg/mL	[24]
Hosseini	2019	Curcumin	-	-	[25]
Azevedo	2014	Polyethyleneimine	Glutaraldehyde	24 mg/L	[26]
Barabadi	2021	AgNPs *	Penicillium chrysogenum	21 g/mL	[27]
Hetta	2021	AgNPs *	Polyvinylpyrrolidone	8 ± 4 µg/mL, 15 ± 4 µg/mL, 19 ± 6 µg/mL	[16]
Salunke	2014	Silver, gold, and bimetallic	Plumbago zeylanica	1,024 µg/ mL	[28]
Li	2021	AgNPs *	Silver nanoparticles were coated with SH-PEG-NOTA as well as loaded by imipenem	-	[29]
Singh	2018	AgNPs *	-	2 mg/mL	[30]
Behdad	2020	AgNPs *	Acroptilon repens	3.9 to 250 µg/mL	[31]
Saleh	2021	ZnONPs **	-	31 - 250 µg/mL	[32]
Madhi	2020	Chitosan and AgNPs *	Ciprofloxacin, Gentamicin	-	[33]
Saleh	2021	ZnONPs **	*Bacillus subtilis*		[34]
Madhi	2020	Chitosan and AgNPs *	Ciprofloxacin, gentamicin		[35]

*** AgNPs - Silver nanoparticles, ** ZnONPs - Zinc oxide nanoparticles.**

ed the growth of *A. baumannii* with MICs ranging from 4 to 25 µg/mL. In addition, AgNPs decreased the expression of *kpsMII*, *afa/draBC*, *bap*, *OmpA*, and *csuA/B* genes. Therefore, it was found that reducing the transcription level of important virulence and biofilm genes stopped the intensity of bacterial proliferation and biofilm formation [16]. Antibiofilm activities of silver, gold, and bimetallic nanoparticles from the Plumbago zeylanica were evaluated in a study by Salunke et al. The results showed that these nanoparticles showed antibiofilm activity against *E. coli*, *A. baumannii*, *S. aureus*. As AgNPs inhibited biofilm in the range of 96% - 99%, and AgAuNP inhibited from 93% to 98% in biofilm. AgNPs destroyed about 88% of the biofilm in *A. baumannii* [28]. Li et al. introduced IPM@AgNPs-PEG -NOTA nanocomposite (silver nanoparticles were coated with SH-PEG-NOTA and loaded by imipenem) as a strong antibacterial agent that can prevent the formation of biofilms by reducing OmpA expression levels [29]. In the study of Singh et al., AgNPs with a minimum concentration of 2 mg/mL could remove biofilm. Biofilm removal increased when exposed to a combination of AgNPs and antibiotics (erythromycin, doxycycline, and tetracyclin). These nanoparticles affected the growth of bacteria by distorting cell morphology and making bacteria susceptible to being killed by nanoparticles with oxidative stress [30].

In Behdad's study, the efflux pump inhibitory activity of AgNPs was evaluated against MDR *A. baumannii*. The results of Real-Time PCR showed that the expression level of the efflux pump genes *AdeA*, *AdeC*, *AdeS*, *AdeR*, *AdeI*, *AdeJ*, and *AdeK* decreased significantly after treatment with AgNPs. The decrease in the expression level of the output pumps was one of the possible mechanisms of their antibacterial activity against the studied strains [31]. The research conducted by Saleh et al. showed the inhibitory effect of biosynthetic zinc oxide nanoparticles (ZnONPs) on efflux pump genes

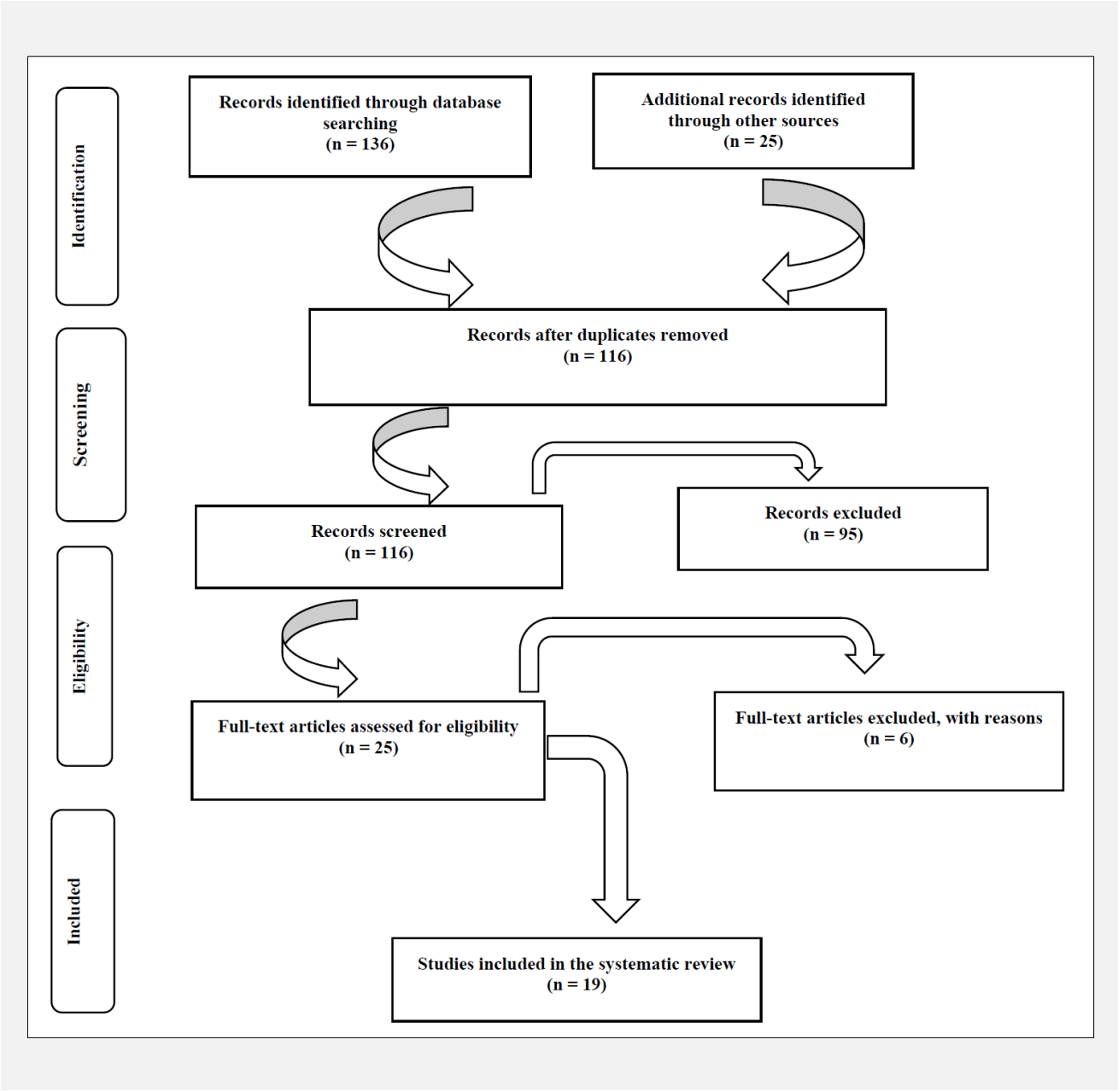

Figure 1. The PRISMA dendrogram for selecting articles for a survey on the inhibitory activity of nanoparticles against *A. baumannii.*

(AdeA, AdeC) expression in clinical *A. baumannii* was investigated. After treatment with ZnONPs, *AdeA* and *AdeC* gene expression decreased in 80% and 40% of the isolates, respectively [32]. Saleh et al. in their study evaluated the effect of ZnONPs derived from *Bacillus subtilis* on the expression of efflux pump genes (*AdeB* and *AdeRS*) in *A. baumannii*. The results showed that the antibacterial properties of ZnONPs increased the expression of *AdeRS* and decreased the expression of *AdeB* in 40% of isolates [34].

Application of nanoparticles

Antimicrobial mechanisms of nanoparticles include the induction of oxidative stress, the release of metal ions, and non-oxidative mechanisms. In nanotechnology, there are new prospects for developing new formulations based on different types of nanoparticles with different sizes and shapes and outstanding antimicrobial properties. Nanoparticles may be a promising solution because they act as carriers of antibiotics and natural antimicrobial compounds in addition to inhibiting bacteria. In addition to this, the unique characteristics of

nanoparticles sometimes increase resistance to antibiotics. For example, the emergence of MDR strains due to plasmid transfer by nanoparticles to bacteria was reported [40]. Also, sometimes nanoparticles may be toxic to cells and cause hemolysis and interfere with blood coagulation. The toxicity of silver nanoparticles for cell lines has been reported in many studies. Deposition of nanoparticles can also lead to serious damage to various organs such as the liver, lung, and spleen. The cost of producing and using nanoparticles is sometimes higher than routine drugs. Arikayce (Insmed) can be mentioned among antibacterial drugs of nanoparticles [41]. Therefore, considering the side effects of nanoparticles after synthesis and determining their antimicrobial activity, cytotoxicity, and side effects *in vivo* conditions should be evaluated, which requires extensive research.

CONCLUSION

The present study shows that nanoparticles have a potential and significant effect in inhibiting biofilm and efflux pumps in *A. baumannii* isolates, which, in the light of the increasing prevalence of antibiotic resistance and the importance of *A. baumannii* as a main cause of nosocomial infection, should be considered by researchers. In addition, it shows the need for more research in evaluating the benefits and harms of nanoparticles as a suitable alternative to antibiotics. It is hoped that in future studies and by conducting more research, we will be able to find the biological and pharmacokinetic aspects of the drug of these nanoparticles in removing *A. baumannii*-producing biofilm and efflux pumps.

Declaration of Interest:
None.

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