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Antimicrobial activity of apramycin formulated with Dextran-based Single Chain Polymeric Nanoparticles against Acinetobacter baumannii

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Abstract

The global emergence of antimicrobial resistance has driven the development of novel strategies for the treatment of bacterial infection. In this context, Dextran-based Single-Chain Polymeric Nanoparticles (DXT-SCPN) have been developed as antibiotic delivery systems for the treatment of pulmonary infections. A series of DXT-SCPN with different physicochemical properties, namely varying degrees of substitution: low (L-DS_{apry}), medium (M-DS_{apry}), and high (H-DS_{apry}) and different crosslinking degrees: medium (M-DS_{apry}) and high (H-CL_{apry}), were formulated with apramycin to evaluate the *in vitro* activity against eleven *Acinetobacter baumannii* clinical isolates. Minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and Time-Kill kinetic (TKK) assays were performed to assess the antibacterial activity of the formulated apramycin in comparison with the free drug. Overall, no statistically significant differences were observed among the antimicrobial activity of free and formulated apramycin. Nevertheless, a slight trend towards higher activity in the case of M-DS_{apry} and H-CL_{apry} was observed.

Introduction

Antibiotic resistance has emerged as a major global health threat in the 21st century. Associated in 2021 with approximately 5 million deaths, antimicrobial resistance (AMR) is estimated to cause up to 40 million deaths annually by 2050 (1).

Acinetobacter baumannii, a Gram-negative bacterium that belongs to the multi-drug resistant ESKAPE group (2), is a primary cause of lower respiratory tract infections (LRTI), including hospital- and community-associated pneumonia (3). Additionally, *A. baumannii* can cause other less frequent serious infections as meningitis, endocarditis and wound infections (4). Between 2010 and 2021, *A. baumannii* was estimated to cause more than 300,000 deaths annually (5).

Resistance to conventional aminoglycosides, such as gentamicin, tobramycin and amikacin has been reported in *A. baumannii* (6). Aminoglycosides resistance is mediated through several mechanisms: aminoglycosides-modifying enzymes (AMEs) such as acetyltransferases, phosphotransferases and nucleotidiltransferases; methylation of the 16S rRNA by methyltransferases (RMTases) which affect to the enzymatic binding site of the aminoglycoside enzyme (6) or efflux pumps (7). *A. baumannii* has also developed resistance to other families of antibiotics as carbapenems, fluoroquinolones, cephalosporins and polymyxins increasing the difficulty of treating its infections (8).

Apramycin, an aminoglycoside primarily used in veterinary medicine, has a distinct molecular structure that prevents its inactivation by AMEs (9) and has demonstrated lower ototoxicity than the other aminoglycosides (10). Due to the increasing incidence of AMR, this antibiotic has been proposed as an alternative treatment of critical Gram-negative and Gram-positive bacterial infection (9, 11). Phase I clinical trials have demonstrated that apramycin is safe and well tolerated at the predicted efficacious dose (30 mg/kg once daily) (12). By 2026, phase II clinical trials have not been carried out and there is not a clinical breakpoint established for in vitro susceptibility testing.

Given the growing antibiotic resistance of *A. baumannii* and the safety and good *in vitro* activity of apramycin, there is an urgent need to advance its use in humans, including inhaled delivery for low respiratory tract

infections (LRTI). In fact, apramycin has been used against carbapenem-resistant *A. baumannii* in lung infections as alternative to colistin (13).

The use of nanoparticles (NPs) has gained importance in the last few years in the treatment of multidrug resistant pathogens (14). It has been demonstrated that Dextran-based Single Chain Polymeric Nanoparticles (DXT-SCPN)(15) could be used as vehicle for antimicrobial molecules at physiological pH. In fact, DXT-SCPN (with a DS -degree of substitution of the nanoparticle which determine the amount of antibiotic that can interact with the nanoparticle- of 50% and CL -indicating the compaction of the nanoparticle - of 5%) has been used to encapsulate the antimicrobial peptide SET-M33 improving the lung residence time of the peptide via aerosol (16). In another study, this encapsulating technology was used to combine tobramycin and DNAse I by electrostatic interactions enhancing the activity of both, tobramycin and DNase I against *Pseudomonas aeruginosa* by improving the penetration into the biofilm compared to the free antibiotic (17). Furthermore, DXT-SCPN has been combined with apramycin and tested against *K. pneumoniae* showing the same effect than free apramycin (18).

Previously, we synthesized four different nanoparticles by varying the reaction condition (amount of reactants) ; three of them with different DS: Lower-DS (L-DS 25%; CL 5%); Medium -DS (M-DS 50%; CL 5%); Higher-DS (H-DS 75%; CL 5%). With the increase of DS, an increase in the amount of apramycin that interact with the nanoparticle was observed. And one of them with an higher CL degree, increasing the nanoparticle compaction: H-CL (DS 50%; CL 15%) (19) .

The aim of this study was to analyse the impact of the DS and CL modifications in the antimicrobial activity of apramycin against *A. baumannii*. For this reason, the Minimal Inhibitory Concentration (MIC), Minimal Bactericidal Concentration (MBC) and Time-Kill Kinetics (TKKs) were determined for the different formulations of DXT-SCPN with apramycin and compared to the activity of free apramycin.

Materials and Methods

Isolates

Between 2010 and 2024 there were 336 cases of *A. baumannii* infections diagnosed by culture at University Hospital Donostia, north of Spain. Urine infections (112 cases) were the most frequent followed by respiratory (77 cases) and wound infections (66 cases). Only 20 isolates were collected from invasive diseases: 18 from blood and two from CSF samples. Eleven isolates were arbitrarily selected for this study: 6 gentamicin susceptible (arbitrarily named Ab1S, Ab2S, Ab3S, Ab4S, Ab5S, Ab6S) and five gentamicin-resistant (Ab7R, Ab8R, Ab9R, Ab10R and Ab11R).

Whole genome sequencing

The genome of the 11 clinical isolates was sequenced using Illumina technology. Genomic DNA was purified using Qiagen columns (QIAamp DNA blood kit, QIAGEN, Hilden, Germany). DNA was quantified using Qubit (Thermofisher Scientific, Waltham, MA, USA) and libraries prepared using the Illumina DNA Prep kit (Illumina, San Diego, CA, USA). Sequencing was performed on a MiSeq platform. Assemblies were conducted using

Unicycler (Version 0.5.1) using the software tools available at the European Galaxy server (https://usegalaxy.eu/). Sequence types (ST) and clonal complexes (CC) were identified by querying assembled genomes on the MLST web page (https://pubmlst.org/organisms/acinetobacter-baumannii). Aminoglycosides resistance genes were identified using ResFinder (version 4.7.2) with default parameters. The assembled genomes were deposited in Genbank as BioProject ID PRJNA1449956.

Formulation of DXT-SCPN with apramycin.

The nanoparticles formulated with apramycin were prepared as previously described (18). Briefly, DXT-SCPNs were dispersed in distilled water under magnetic stirring (150 r.p.m.) for 1 h. Separately, apramycin was dissolved in water under magnetic stirring for 1 h. The pH of each solution was adjusted at 7.0 by adding NaOH or HCl 0.1M and HCl 1M in the case of DXT-SCPN and apramycin, respectively. Finally, distilled water was added until the final concentration of each solution was 50 mg/mL. DXT-SCPN was added to apramycin solution (the ratio DXT-SCPN:Apry depended on the wt% for each DS and CL to reach neutralization: L-DS 20 wt%; M-DS/L-CL and H-CL: 30 wt% and H-DS: 40 wt%). Nanoformulation were freeze-dried and kept at 4ºC until further research.

Determination of Minimal Inhibitory and Bactericidal Concentration.

The Minimal Inhibitory Concentration (MIC) for each nanoformulation was determined using the broth microdilution method as described by the European Committee on Antimicrobial Susceptibility Testing (EUCAST; https://www.eucast.org). *Escherichia coli* ATCC 25922 was used as a control. Stock solutions of free and formulated apramycin were prepared and adjusted to 128 mg/L in cation-adjusted Mueller Hinton broth (MHB) based on apramycin content. Two-fold serial dilutions were performed to prepare 50 µL of apramycin dilutions. Then, 50 µL of the bacterial inoculum adjusted to ~ 10^6 CFU/mL (a 1:100 dilution of a McFarland 0.5 standard) were added to each well and incubated for 24 hours at 37ºC with a final apramycin concentration range: 1 to 64 mg/L. MICs were defined as the lowest apramycin concentration at which no visible growth was observed. Growth positive and negative controls were included in each test.

Once MIC was determined, 10 µL of each well was transferred into a blood agar plate and incubated for 24 hours and 37ºC. The lowest concentration which showed non-bacterial growth was defined as the Minimal Bactericidal Concentration (MBC). The same protocol but with a range of 4 to 256 mg/L was used to determine nanoparticles MICs and MBCs without the antibiotic.

Experiments of MIC and MBC was carried out three times in three different days.

Time-Kill Kinetic assay

The activity of free apramycin and in nanoformulations was studied by the Time-Kill Kinetic (TKK) assay. Three gentamicin-susceptible *A. baumannii* clinical isolates (Ab3S, Ab4S y Ab5S) arbitrarily selected were studied. Bacterial suspensions were adjusted by turbidimetry to 0.5 McFarland in saline buffer. A 1:100 dilution was performed in 5 mL of cation-adjusted MHB containing apramycin at concentrations of 4, 8 and 32 mg/L. Tubes were incubated under agitation and 37 ºC. 100 µL were removed at 0, 2, 4, 6, 12 and 24 h and the

quantification of viable bacteria was performed transferring ten-fold dilution into an agar blood plate as described (20).

Statistical analyses

ANOVA was carried out with TKK values to analyse the significant differences respect to the free apramycin. A significance of $p < 0.05$ was considered as statistically significant.

Ethics statement

The Research Ethics Committee of the Gipuzkoa Health Area (Comité de Ética de la Investigación del Área Sanitaria de Gipuzkoa, CEI; protocol code JOZ-APRA-2026-01) ruled that the present work falls outside its material and legal scope of application and therefore does not require mandatory ethical approval or continuous monitoring by the Committee, based on Article 1 of the Spanish Biomedical Research Act (Law 14/2007) and Recital 26 of Regulation (EU) 2016/679 (GDPR). Accordingly, the need for informed consent was deemed unnecessary by the Research Ethics Committee of the Gipuzkoa Health Area, as the study is a purely *in vitro* microbiological investigation using irreversibly anonymized historical bacterial isolates and does not involve clinical, epidemiological, or identifiable patient data. All procedures were conducted in accordance with the relevant institutional guidelines and applicable Spanish legislation, as confirmed by the Research Ethics Committee of the Gipuzkoa Health Area.

Results

Genotyping and aminoglycoside resistance determinants

Clinical isolates selected for this study were collected from different sources and showed different STs (Table 1). Two of the five gentamicin resistant isolates had an apramycin MIC = 16 mg/L and both had the *aph(3')-VI* gene.

Table 1
Characterization of the *Acinetobacter baumannii* clinical isolates included in this study: MLST typing, apramycin MIC (mg/L) and aminoglycoside genes present in each isolate.

Isolates	Source	ST[1]	Apramycin MIC (mg/L)	Aminoglycoside resistance genes[2]
Ab1S	rectal swab	265	4	
Ab2S	blood	203	4	
Ab3S	urine	203	4	
Ab4S	urine	64	8	
Ab5S	blood	150	4	
Ab6S	urine	23	4	
Ab7R	urine	2	4	aac(6')-Ib, aadA1, aph(3'')-Ib, aph(3')-Ia, aph(6)-Id, armA
Ab8R	BAL[3]	2	8	aac(6')-Ib, aadA1, aph(3'')-Ib, aph(3')-Ia, aph(6)-Id, armA
Ab9R	urine	570	16	aac(6')-Ib, aadA1, aph(3')-Ia, aph(3')-VI, aph(3')-VIa, armA
Ab10R	sputum	570	16	aac(6')-Ib, aadA1, aph(3')-Ia, aph(3')-VI, armA
Ab11R	blood	10	> 64	aph(3')-Ia
ATCC25922[4]			4	

1. ST: sequence type 2. aac: aminoglycoside acetyltransferase; aad: aminoglycoside O-nucleotidyltransferase, aph: aminoglycoside phosphotransferase. 3: BAL; bronchoalveolar lavage. ATCC25922: *E. coli* reference strain.

Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC).

Initially, the antimicrobial activity of all the nanoparticles (L-DS, M-DS, H-DS and DS-CL) without apramycin was tested. As expected, no antibacterial activity was observed for any of the nanoparticles alone (all MICs > 256 mg/L).

Five of the six gentamicin-susceptible *A. baumannii* isolates showed an apramycin MIC = 4 mg/L, and the remaining isolate (Ab4S) a MIC = 8 mg/L (Table 2). In formulated apramycin, for L-DS_{apry} a MIC = 8 mg/L was observed for most gentamycin-susceptible isolates. M-DS_{apry} showed a MIC = 4 mg/L except for Ab1S (MIC = 8 mg/L). H-DS_{apry} showed MICs between 4 and 8 mg/L except for isolate Ab1S (MIC = 16 mg/L). The increase in CL (H-CL_{apry}) resulted in MICs close to that of free apramycin.

Two of the five gentamicin-resistant isolates, Ab7R and Ab8R, showed that free and formulated apramycin with MICs of 4–8 mg/L, except for H-DS_{apry} (MIC = 16 mg/L). Ab9R and Ab10R exhibited intermediate MICs

values (between 4–16 mg/L and 16–32 mg/L, respectively) while Ab11R had MICs > 64 mg/L. MBC values were the same as the MICs for most free and formulated apramycin combinations.

Table 2
Minimal inhibitory concentration (MIC; mg/L) and minimal bactericidal concentration (MBC; mg/L) of *A. baumannii* clinical isolates for free and nanoformulated apramycin.

Isolates	Gentamicin	Free Apramycin		L-DS_{apry}[1]		M-DS_{apry}[1]		H-DS_{apry}[1]		H-CL_{apry}[1]	
	MIC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Ab1S	≤ 1	4	4	8	8	8	8	16	16	8	8
Ab2S	≤ 1	4	4	4	4	4	4	8	8	4	4
Ab3S	≤ 1	4	8	8	8	4	4	4	8	4	4
Ab4S	≤ 1	8	8	8	8	4	8	8	8	4	4
Ab5S	≤ 1	4	8	8	8	4	8	8	8	4	8
Ab6S	≤ 1	4	4	8	8	4	8	8	8	4	8
Ab7R	≥ 16	4	4	8	8	4	4	16	16	8	8
Ab8R	≥ 16	8	8	8	8	4	4	8	8	4	4
Ab9R	≥ 16	16	16	8	8	8	16	8	16	8	16
Ab10R	≥ 16	16	16	32	32	8	16	16	32	16	16
Ab11R	≥ 16	> 64	> 64	> 64	> 64	> 64	> 64	> 64	> 64	> 64	> 64
ATCC25922[2]	≤ 1	4	4	8	8	4	4	8	8	4	4

1. Apramycin rate in nanoformulation: L-DS_{apry}: 20 wt%; M-DS_{apry} and H-CL_{apry} 30 wt% H-DS_{apry}: 40 wt%. Data express the median of three experiments.
2. ATCC25922: *E. coli* reference strain.

Time-Kill Kinetics assays

Time-Kill Kinetics at 4 mg/L

Isolate Ab3S exhibited different activity depending on the formulation. Treatment with H-DS_{apry} at 4 mg/L showed a progressive increase of viable bacteria over time (from 10^6 to 10^{11} CFU/mL at 24 h). With free apramycin and L-DS_{apry} after an initial bacteriostatic effect, bacteria regrew to 10^{11} CFU/mL at 24 h (Fig. 1.1A). In contrast, M-DS_{apry} and H-CL_{apry}, initially reduced viable bacteria to 10^3 CFU/mL at 6 h with no growth observed at 12 h. At 24 h, regrowth was observed for M-DS_{apry} whereas H-CL_{apry} maintained its bactericidal activity. The reductions achieved by M-DS_{apry} and H-CL_{apry} at 12 and 24 h were statistically significant compared to free-apramycin (ANOVA test $p < 0.001$) (Figure. 1.1B).

Isolate Ab4S showed, for free apramycin, a reduction of viable bacteria to 10^3 CFU/mL in the first 4 h, no growth at 6 and 12 h, but regrowth occurred at 24 h (Fig. 1.2A). L-DS_{apry} at 4 mg/L showed limited activity, with initial bacterial counts of 10^3 CFU/mL up to 6 h and increases of viable cells at 12 and 24 h. Both, M-DS_{apry} and H-CL_{apry} demonstrated bactericidal activity, with no bacterial growth after 12 h. H-DS_{apry} showed bacteriostatic activity until 6 h, after which counts increased to 10^{11} CFU/mL ($p < 0.005$ at 6 h and $p < 0.001$ at 12 and 24 h).

For isolate Ab5S, an initial decrease of bacterial inoculum until 6 h was observed, more pronounced for free apramycin than for L-DS_{apry} and M-DS_{apry} (from 10^6 CFU/mL to 10^1 CFU/mL and to 10^4 CFU/mL, respectively). At 12 h and 24 h bacterial counts increased, reaching 10^6 CFU/mL for free apramycin and 10^{11} CFU/mL for L-DS_{apry}, and M-DS_{apry}. Nevertheless, the standard deviation bar of free apramycin at 24 h indicated a high deviation, demonstrating a regrowth in two of the three replicates. For H-DS_{apry} at 4 mg/L, a progressive increase of viable bacteria over time was observed, reaching 10^{11} CFU/mL at 24 h. In the presence of H-CL_{apry}, the bacterial population decreased to 10^3 CFU/mL over the first 6 h without subsequent growth at 12 and 24 h ($p < 0.001$ for 24 h compared to free apramycin) (Fig. 1.3B).

Time Kill Kinetics at 8 mg/L

TKK profiles at 8 mg/L differed from those at 4 mg/L, with an improved overall killing for all formulations across isolates Ab3S, Ab4S, and Ab5S (Fig. 2).

For isolate Ab3S (Fig. 2.1A), free apramycin reduced the bacterial population to 10^4 CFU/mL at 2 h, maintained to 6 h, with no growth from 12 h onwards. L-DS_{apry} reduced growth to 10^3 CFU/mL after 4 h, sustained to 24 h. M-DS_{apry} and H-CL_{apry} showed a similar profile as free apramycin; with M-DS_{apry}, however, a regrowth to 10^3 CFU/mL occurred at 24 h. H-DS_{apry} maintained bacterial counts at around 10^6 CFU/mL over 12 h, followed by an increase to 10^{11} CFU/mL at 24 h.

Ab4S TKK profiles (Fig. 2.2A) were similar for all formulations: a progressive decline in bacterial counts in the first 2–4 h with no bacterial growth from 6 h onwards for apramycin, L-DS_{apry} and H-CL_{apry} and from 12 h for H-DS_{apry} and M-DS_{apry}.

For isolate Ab5S (Fig. 2.3A), free apramycin activity was similar to that of isolate Ab3S. L-DS_{apry} and H-CL_{apry} showed no growth from 6 h, and M-DS_{apry} from 12 h. For H-DS_{apry}, a progressive decrease in bacterial counts was observed over time, but complete eradication was not achieved after 24 h.

At 8 mg/L, all apramycin formulations except H-DS_{apry} demonstrated sustained bactericidal activity after 6–12 h, achieving rapid 10^{2-3} CFU/ml reductions with no regrowth after. Higher bacterial counts for H-DS_{apry} compared to free apramycin were statistically significant at 12h ($p < 0.01$) for Ab3S and Ab5S and 24h ($p < 0.001$) just in the case of Ab3S.

Time-Kill Kinetic at 32 mg/L

At 32 mg/L (Fig. 3), free apramycin eradicated the initial bacterial inoculum of 10^6 CFU/mL across the 3 clinical *A. baumannii* isolates within 2 h. Nanoformulations achieved equivalent bacterial activity by 2 h, except for H-DS_{apry} which required 4 h for Ab3S and Ab4S and 6 h for Ab5S. Statistical analyses (See Supporting Information; Figure S1) confirmed significant delays for H-DS_{apry} compared to free apramycin at 2 h for Ab3S and Ab4S and at 6 h for Ab5S.

Discussion

Antibiotic formulation with nanoparticles has been explored to improve their activity through different mechanisms, including enhanced antibiotic delivery and tissue targeting (21). In this study, we evaluated apramycin formulated with a series of DXT-SCPN nanoparticles with different physicochemical features against clinical isolates of *A. baumannii* to determine whether nanofomulations modified apramycin antimicrobial activity.

MIC and MBC values of free and formulated apramycin, ranging between 4 and 8 mg/L for free apramycin, M-DS_{apry} and H-CL_{apry}, indicated that DXT-SCPN nanoparticles did not significantly modify, within one-double dilution, apramycin´s antimicrobial activity. Nevertheless, it was observed a slight trend towards higher MICs (lower activity) in the case of L-DS_{apry} and H-DS_{apry}. This contrasts with other type of nanoparticle-aminoglycoside combinations, as silver nanoparticles, that showed a synergistic effect with aminoglycosides (22, 23). Although no *in vitro* potentiation was observed, polymeric nanoparticles could be used as a novel delivery vehicle aiming to improve apramycin pharmacokinetics as retention and biodistribution in the pulmonary parenchyma, without compromising antibiotic efficacy (24).

Apramycin has been shown to be active against gentamicin-resistant clinical isolates of *A. baumannii*, because the mechanisms of resistance to apramycin differs from the mechanisms of resistance to other aminoglycosides. (9). Considering a wild-type cut-off value of 16 mg/mL for apramycin (11), two of five gentamicin-resistant isolates remained susceptible to apramycin, both in free and nanoformulated forms. These findings support a potential role for apramycin in the treatment of multidrug-resistant *A. baumannii* infections, including isolates resistant to conventional aminoglycosides (9). In fact, some research supports the apramycin efficacy against carbapenem-resistant or aminoglycosides-resistant *A. baumannii* alone (13) or in combination with colistin (25). The two apramycin resistant isolates with a MIC = 16 mg/L had the phosphotransferase aph(3')-VI gene, not present in gentamycin-resistant apramycin-susceptible isolates, suggesting its possible role for resistance. The apramycin-highly resistant isolate with a MIC > 64 mg/L did not have the aac(3)-IV gene, that has been related to apramycin resistance (26), what points out to other mechanism of resistance.

Low apramycin concentrations (4 and 8 mg/L) were specifically chosen in TKK experiments to try to resolve subtle differences between nanoformulations and free apramycin while a high concentration (32 mg/L) was chosen to observe their bactericidal effect. There was a trend towards lower MICs and more rapid killing for high crosslinking (H-CL_{apry}) nanoparticles and the opposite effects for highly functionalized nanoparticles (H-DS_{apry}). In line with previous TKKs reports (25), free apramycin displayed a bactericidal effect at 4 mg/L (in two of three isolates) and bactericidal effect in all isolates at 8 mg/L. In the case of H-CL_{apry}, a bactericidal effect was demonstrated in the three isolates after 12 hours at both studied concentrations (4 and 8 mg/L).

This result was similar to other studies in which aminoglycosides, such as tobramycin or streptomycin, were encapsulated in different polysaccharidic system showing a similar antibiotic efficacy respect to the free antibiotic (27). H-DS_{apry} showed bactericidal activity at 32 mg/L in the three isolates studied, but not at 4 and 8 mg/L. The other nanoformulations, L-DS_{apry} and M-DS_{apry}, showed an intermediate antimicrobial activity with a bactericidal activity at 8 mg/L. These results are consistent with other research in which M-DS_{apry} was tested against *Klebsiella pneumoniae* showing bactericidal activity for free and formulated apramycin at 8 mg/L, after 12 hours (9).

The small differences in the antimicrobial performance among the nanoformulation may be attributed to different nanoparticles characteristics. One of them is the type of interactions of the nanoparticles with the antibiotic. The primary type of interaction between DXT-SCPN and apramycin is electrostatic; nevertheless, stronger interactions, such as Van der Waals forces and hydrogen bonding, were observed in aqueous media, particularly for H-CL_{apry} (19). These stronger interactions could contribute to the slightly enhanced activity of H-CL_{apry} due to protection of the drug from degradation or to an increase in the antibiotic release time (28). Previous studies have shown that higher crosslinking could improve the antibacterial efficacy (29). For instance, in the case of chitosan nanoparticles, a significant correlation between the compaction of the nanoparticles and antibacterial activity was observed, with crosslinked polymers exhibiting higher antibacterial activity than non-crosslinked chitosan (30). Another example was reported with hypercrosslinked polymers that showed L-borneol dose-dependent antibacterial activity, unlike the free molecule, which showed virtually no activity (31). Other characteristic that can affect the antimicrobial activity of nanoparticles is the formation of aggregates. Previous studies demonstrated that the presence of aggregates decreased the antimicrobial activity of silver nanoparticles (32). H-DS_{apry} formulation showed the highest aggregates formation (higher size) (19) and a diminish apramycin efficacy compared to the other nanoformulations. This effect was observed in other polymeric systems, such as chitosan nanoparticles, in which higher size was related with a decrease of antibacterial efficacy due to the poor penetration into the bacteria (33).

One of the limitations of this study us that it included a relatively low number of *A. baumannii* clinical isolates, which should be taken into account when considering the generalizability of the findings. Nonetheless, the data herein obtained add information about the antimicrobial capabilities of nanoformulated apramycin. Further studies, including *in vivo* experiments will help to validate these results and to better characterize both the pharmacokinetic properties and antimicrobial activity of nanoformulated apramycin.

In conclusion, apramycin showed good antimicrobial activity against different clinical isolates of *A. baumannii*, a frequent human pathogenic bacterium that it is becoming increasingly multiresistant. A bactericidal effect at 8 mg/L was observed for most clinical isolates, even against two gentamicin resistant isolates. No alteration in the antimicrobial activity was observed in the apramycin nanoformulations with different DXT-SCPN. It was observed that H-CL_{apry} nanoformulation improved the antibiotic activity at 4 mg/L while H-DS_{apry} decrease the efficacy of the antibiotic at the same concentrations in the studied condition. The administration advantages that nanoformulations can add to the antimicrobial activity profile of apramycin make this combination a promising alternative for new therapeutic options.

Declarations

Author contribution statement

J.C.I. carried out the experimental part. J.C.I. and J.M.M wrote the main manuscript text. J.C.I, J.M, D.D, I.L. and J.M.M reviewed and revised the manuscript.

Data availability y statement

The assembled genomes were deposited in Genbank as BioProject ID PRJNA1449956. Furthermore, all data supporting the findings of this study are available upon request.

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Competing interests

The authors declare no competing financial interest.

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Figures

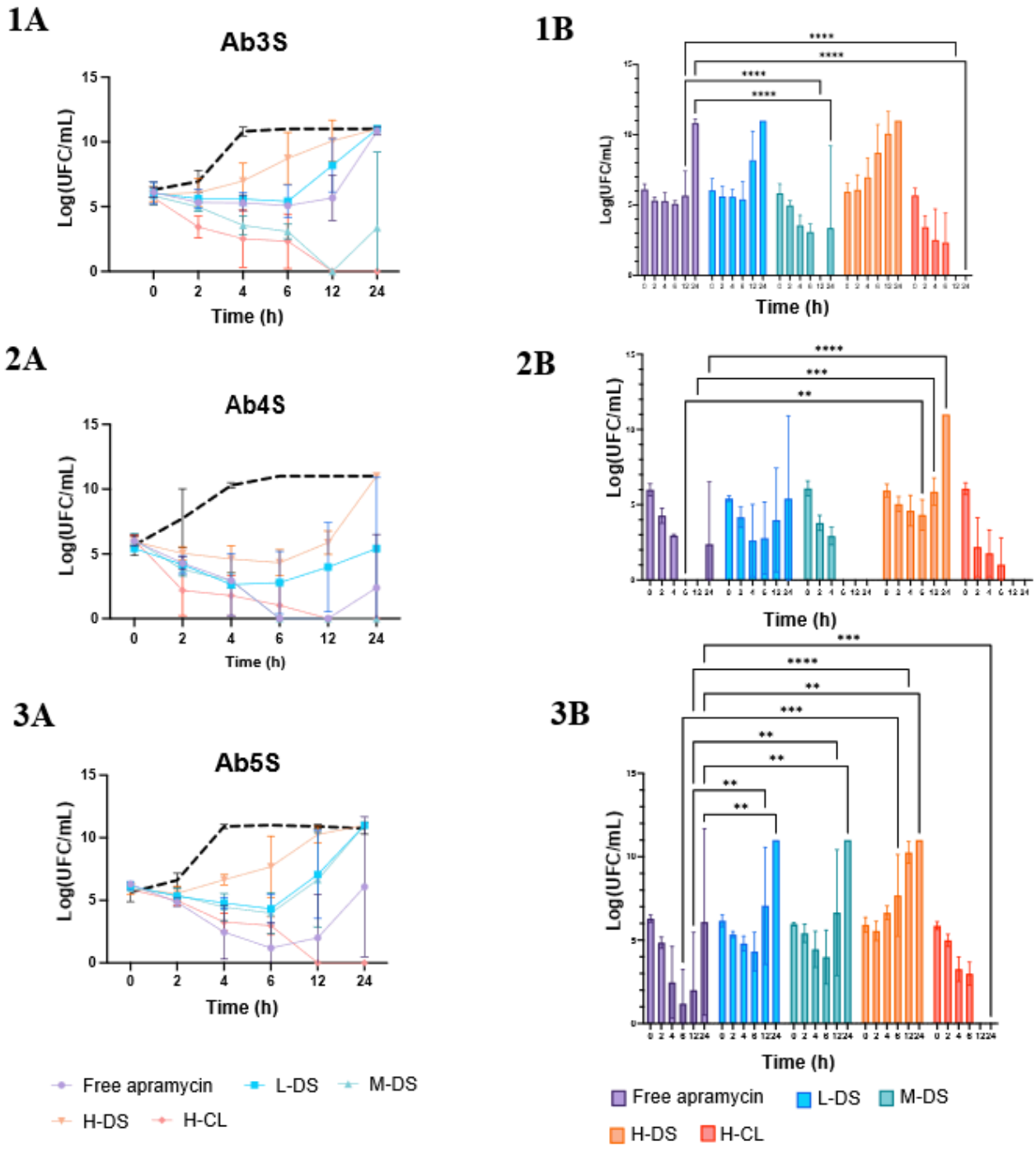


Figure 1

Time-Kill kinetics (TKK) (A) and significant differences respect to free apramycin by ANOVA (B) of three A. baumannii clinical isolates treated with free and formulated apramycin at 4 mg/L. 1A and 1B: isolate Ab3S. 2A and 2B: Isolate Ab4S. 3A and 3B: Isolate 5AbS. The dotted line represents the TKK without antibiotic. Bar error shows the standard deviation of three individual experiments. Significant differences respect free apramycin by ANOVA: * $p<0.05$, ** $p< 0.01$, *** $p<0.005$, **** $p<0.001$.

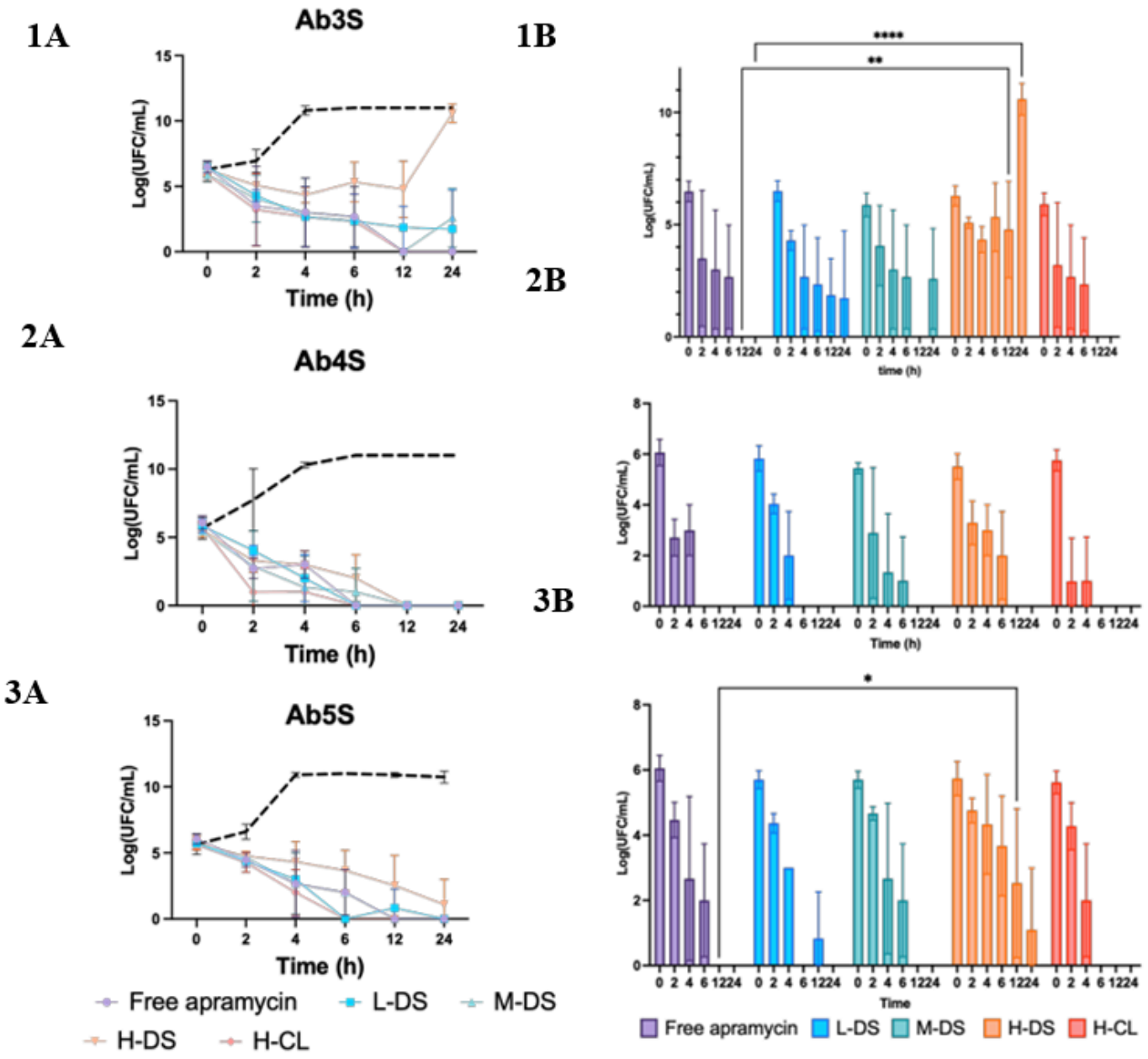


Figure 2

Time-Kill kinetics (TKK) (A) and significant differences respect free apramycin by ANOVA (B) of three A. baumannii clinical isolates treated with free and formulated apramycin at 8 mg/L. 1A and 1B: isolate Ab3S. 2A and 2B: Isolate Ab4S. 3A and 3B: Isolate 5AbS. The dotted line represents the TKK without antibiotic. Bar error shows the standard deviation of three individual experiments. Significant differences respect free apramycin by ANOVA: * p<0.05, ** p< 0.01, *** p<0.005, **** p<0.001.

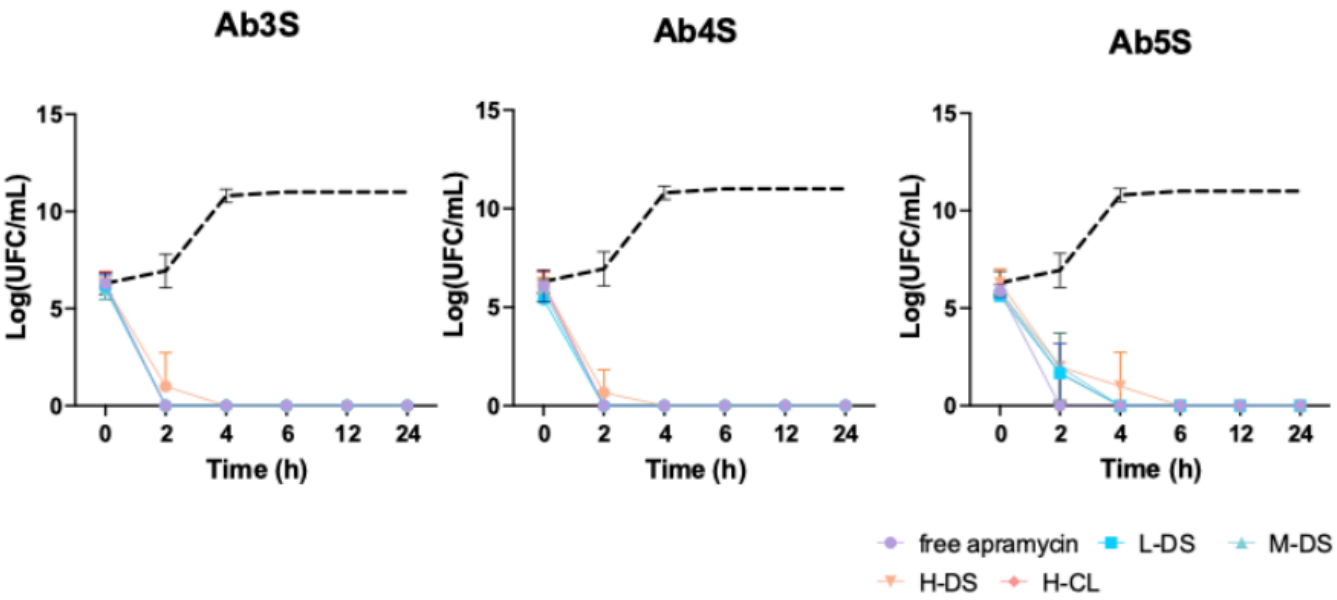


Figure 3

TKK of free and formulated apramycin against *A. baumannii* at 32 mg/L. The dotted line represents the bacteria growth without antibiotic. Bar error shows the standard deviation of three replicates in three different days.