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Development, Characterization, And In Vitro Evaluation Of Phospholipid-Based Liposomes Encapsulating Linezolid For Targeted Therapeutic Management Of Gram-Positive Bacterial Infections

Uttam Kumar[1], Mrityunjaya Ullagaddi[2], Satinder Kumar[3*], Priyanka[4], Asokan B.R.[5], Ashutosh Pathak[6], Naveen Sharma[7], Harsh Goswami[8]

[1]Department of Pharmaceutical Analysis, KLE College of Pharmacy, Rajajinagar, Bengaluru. KLE Academy of Higher Education and Research, Belagavi. 590010, Karnataka, India.

[2]Department of Pharmacology, KLE College of Pharmacy, Rajajinagar, Bengaluru. KLE Academy of Higher Education and Research, Belagavi. 590010, Karnataka, India.

[3]Department of Pharmaceutics, School of Pharmaceutical Sciences, RIMT University, Mandi Gobindgarh, Punjab Pin- 147301.

[4]Department of Pharmaceutical Chemistry, College of Pharmacy, RIMT University, Mandi Gobindgarh, Punjab Pin – 147301.

[5]Department of Pharmacology TSRMMCH & RC, Irungalur, Trichy, 621105, The Tamil Nadu Dr. MGR, Medical University, Chennai.

[6]Department of Pharmacy Practice, Teerthanker Mahaveer College of Pharmacy, Teerthanker, Mahaveer University, Moradabad UP, India Pin- 244001.

[7]Department of Pharmacy, Chandigarh Pharmacy College, Chandigarh Group of Colleges Jhanjeri, Mohali, Punjab, India - 140307.

[8]Raj Kumar Goel Institute of Technology (Pharmacy), Ghaziabad, Uttar Pradesh, India, 201013

Corresponding Author: Satinder Kumar 3*, Department of Pharmaceutics, School of Pharmaceutical Sciences, RIMT University, Mandi Gobindgarh, Punjab Pin- 147301. Email ID: satinder1000.johar@gmail.com

Abstract

The present study aimed to design, optimize, and evaluate liposomal formulations of Linezolid for improved antibacterial efficacy and targeted drug delivery. Linezolid, a potent oxazolidinone-class antibiotic, was encapsulated in liposomes using the thin-film hydration technique followed by sonication. A series of five formulations (LZL1–LZL5) were prepared by varying the ratios of phosphatidylcholine and cholesterol to optimize particle size, polydispersity index (PDI), zeta potential, entrapment efficiency, and drug release profile. Among the developed formulations, LZL3 demonstrated optimal characteristics with a particle size of 164.3 ± 2.3 nm, PDI of 0.247, zeta potential of –27.6 ± 0.9 mV, and entrapment efficiency of 81.56 ± 2.5%. Transmission Electron Microscopy confirmed spherical and uniform vesicle morphology. In vitro release studies revealed a sustained drug release from LZL3 (88.56 ± 1.89% at 12 h), while antibacterial activity assays showed significant zones of inhibition against Staphylococcus aureus and Escherichia coli, comparable to the free drug. These findings suggest that liposomal encapsulation of Linezolid enhances its delivery and therapeutic potential while offering controlled release and improved stability. The study supports the use of liposomes as an effective platform for targeted antibiotic therapy, potentially reducing dosing frequency and systemic side effects.

Keywords: *Linezolid, Liposomal drug delivery, Targeted antibacterial therapy, Encapsulation efficiency, Controlled drug release, Nanocarrier systems.*

1. INTRODUCTION

The rise of antimicrobial resistance (AMR) has become one of the most pressing global health concerns of the 21st century. The overuse and misuse of antibiotics have accelerated the development of resistant bacterial strains, diminishing the efficacy of many conventional antibiotics. In this context, the development of advanced drug delivery systems that enhance the therapeutic efficacy of existing antibiotics, reduce side effects, and allow for targeted action has emerged as a promising strategy to combat AMR. One such promising approach is the use of nanocarrier-based drug delivery systems, particularly liposomes(Brinkac et al., 2017; Catalano et al., 2022; Mehrotra et al., 2023; Qiao et al., 2018).

Liposomes are spherical vesicles composed of one or more phospholipid bilayers enclosing an aqueous core. Since their discovery in the 1960s, liposomes have been widely investigated and applied in the

pharmaceutical field due to their biocompatibility, biodegradability, and ability to encapsulate both hydrophilic and lipophilic drugs. They offer several advantages over conventional dosage forms, including protection of encapsulated drugs from degradation, improved pharmacokinetics and pharmacodynamics, reduced toxicity, and the potential for targeted delivery. These properties make liposomes an ideal carrier for antibiotics, especially for drugs that require sustained release or targeted action at the site of infection(Ahmed et al., 2023; Franco-Gonzalez et al., 2022; Marchianò et al., 2020).

Linezolid, a synthetic antibiotic belonging to the oxazolidinone class, has gained significant attention in the treatment of infections caused by Gram-positive bacteria, including drug-resistant strains such as methicillin-resistant Staphylococcus aureus (MRSA) and vancomycin-resistant Enterococcus (VRE). It acts by inhibiting bacterial protein synthesis through binding to the 50S ribosomal subunit, preventing the formation of the initiation complex. Despite its effectiveness, Linezolid is associated with several limitations when administered orally or intravenously, including poor solubility, limited bioavailability, and potential for adverse effects such as thrombocytopenia and optic neuropathy with prolonged use (Crass et al., 2019; Hashemian et al., 2018; Miller et al., 2023; Zahedi Bialvaei et al., 2017). Therefore, reformulating Linezolid into a controlled-release delivery system like liposomes could significantly enhance its therapeutic index (Ahsan et al., 2024; Chahar et al., 2023; Vairo et al., 2023).

Encapsulation of Linezolid into liposomal carriers can potentially overcome the aforementioned drawbacks by modifying the drug's release profile, enhancing its bioavailability, and reducing systemic exposure. Liposomes can also facilitate localized drug delivery, especially to infected tissues where the permeability of blood vessels is increased due to inflammation. This targeted delivery approach not only enhances drug accumulation at the infection site but also minimizes off-target toxicity and adverse reactions, making it highly suitable for chronic or high-dose antibiotic therapies. The process of liposome preparation, however, must be carefully optimized to achieve desirable characteristics such as uniform particle size, high drug entrapment efficiency, and long-term stability. Parameters such as the lipid-to-cholesterol ratio, hydration conditions, sonication time, and the method of preparation significantly influence the physicochemical properties of the final formulation. Among various techniques available for liposome preparation, the thin-film hydration method is widely preferred due to its simplicity and effectiveness in producing multilamellar vesicles, which can subsequently be converted into small unilamellar vesicles via sonication or extrusion (Conradie et al., 2022; Rao et al., 2020).

Particle size is a critical attribute of liposomal formulations as it affects the biodistribution, cellular uptake, and clearance of the particles from the systemic circulation. Smaller vesicles, particularly those below 200 nm, are generally favored for intravenous administration as they can evade rapid clearance by the reticuloendothelial system (RES) and exhibit prolonged circulation time. Additionally, the zeta potential of liposomes influences their colloidal stability; liposomes with high surface charge tend to repel each other, thereby reducing aggregation. Another crucial parameter is the polydispersity index (PDI), which provides a measure of the uniformity of particle sizes in a formulation. A low PDI (<0.3) indicates a narrow size distribution and better reproducibility. Entrapment efficiency is another important factor that determines the amount of drug successfully encapsulated within the liposomes. High entrapment ensures a higher payload, which is especially desirable in targeted therapy to deliver an effective dose to the infected site (Conradie et al., 2022; Rao et al., 2020). The encapsulated drug must also be able to release in a controlled manner to maintain therapeutic drug levels over an extended period. In vitro drug release studies are typically conducted using dialysis methods to assess the release kinetics, which can further be analyzed using various mathematical models such as zero-order, first-order, Higuchi, and Korsmeyer-Peppas models to understand the underlying release mechanisms. In addition to physicochemical characterization, it is essential to evaluate the biological performance of the liposomal formulations. Antibacterial activity testing, commonly performed using the agar well diffusion method, helps determine whether the encapsulated drug retains its bioactivity and whether liposomal encapsulation enhances or maintains its therapeutic potential. Such assays can provide a comparative understanding of the effectiveness of liposomal formulations versus free drug solutions (Ahmed et al., 2023; Ahsan et al., 2024; Brinkac et al., 2017; Zahedi Bialvaei et al., 2017).

The present study was designed to formulate and optimize Linezolid-loaded liposomes using the thin-film hydration method. A total of five formulations (LZL1 to LZL5) were developed by varying the

concentrations of phosphatidylcholine and cholesterol to assess their effect on particle size, PDI, zeta potential, entrapment efficiency, and in vitro release profile. The optimized formulation was further subjected to morphological evaluation using Transmission Electron Microscopy (TEM) and in vitro antibacterial testing against *Staphylococcus aureus* and *Escherichia coli.*

By integrating physicochemical characterization with functional evaluation, this study aims to provide a comprehensive understanding of the potential of liposomal carriers for enhancing the delivery and efficacy of Linezolid. The ultimate goal is to develop a stable, effective, and targeted liposomal formulation that could serve as a platform for improving the treatment outcomes of bacterial infections, particularly those involving resistant pathogens. In summary, the development of a liposomal drug delivery system for Linezolid addresses the need for improved therapeutic strategies in the fight against antimicrobial resistance. With careful design and optimization, such systems can not only improve the pharmacological profile of the drug but also contribute to more efficient and safer infection management. This research, therefore, represents a critical step toward the advancement of nanotechnology-based antimicrobial therapies.

2. MATERIAL AND METHODS

2.1. Material

Linezolid was obtained as a gift sample from a reputed pharmaceutical company (Zing Pharma, Ambala, India). Soybean phosphatidylcholine (SPC) and cholesterol were procured from Sigma-Aldrich (St. Louis, MO, USA). Chloroform and methanol of analytical grade were purchased from Merck Chemicals (India) and used for lipid dissolution. The dialysis membrane (molecular weight cut-off 12–14 kDa) required for in vitro release studies was sourced from HiMedia Laboratories Pvt. Ltd., India. All other chemicals and solvents used in the study were of analytical grade and used without further purification.

2.2. Preparation of Linezolid-Loaded Liposomes

Linezolid-loaded liposomes were prepared using the classical thin-film hydration technique followed by probe sonication to reduce particle size and ensure uniformity. The formulations (LZL1 to LZL5) were developed by varying the concentrations of phosphatidylcholine and cholesterol, while keeping the drug amount, organic phase volume, and hydration conditions constant. The detailed composition of each formulation is presented in Table 1. In this process, accurately weighed amounts of Linezolid, phosphatidylcholine, and cholesterol (as per Table 1) were dissolved in a 10 mL organic solvent mixture of chloroform and methanol in a 2:1 (v/v) ratio in a 100 mL round-bottom flask. This mixture ensured complete dissolution of all lipid components and the drug, promoting homogeneous distribution in the lipid bilayer during film formation. The organic solvent was evaporated under reduced pressure at 40 ± 2°C using a rotary vacuum evaporator (Rotavapor) at a rotation speed of 90 rpm, forming a thin and uniform lipid film on the inner wall of the flask. After complete solvent removal, the dry lipid film was subjected to vacuum desiccation for 2 hours to eliminate residual solvent traces, which could affect vesicle formation and drug stability. Subsequently, the dry lipid film was hydrated using 10 mL of phosphate-buffered saline (PBS, pH 7.4) preheated to 37°C. The hydration was carried out under continuous rotation at 60 rpm for 30 minutes to form multilamellar vesicles (MLVs). The flask was gently agitated to facilitate complete dispersion of the lipid film into the aqueous medium. The resulting milky dispersion was then subjected to probe sonication using a sonicator (e.g., Branson Ultrasonics) for 5 minutes with intermittent pulsing (30 seconds on, 10 seconds off) in an ice bath to avoid thermal degradation. This step led to the conversion of MLVs into small unilamellar vesicles (SUVs) with reduced particle size, better homogeneity, and improved surface characteristics. The prepared liposomal dispersions were stored in amber-colored vials at 4°C for further characterization and evaluation (Chaurasiya et al., 2022; Guimarães et al., 2021; Liu et al., 2022).

Table 1: Composition of Linezolid-Loaded Liposomal Formulations

Ingredients	LZL1	LZL2	LZL3	LZL4	LZL5
Linezolid (mg)	50	50	50	50	50
Phosphatidylcholine (mg)	100	150	200	200	250
Cholesterol (mg)	25	30	40	50	60

Chloroform:Methanol (2:1 v/v, mL)	10	10	10	10	10
Hydration Medium (PBS pH 7.4, mL)	10	10	10	10	10
Sonication Time (min)	5	5	5	5	5

2.3. Optimization of Formulation

To identify the most suitable liposomal system for the targeted delivery of Linezolid, a systematic formulation optimization approach was undertaken. A total of five formulations (LZL1 to LZL5) were developed by varying the ratios of phosphatidylcholine and cholesterol, while maintaining a constant drug concentration. This variation allowed the investigation of the effects of lipid composition on key physicochemical characteristics such as particle size, polydispersity index (PDI), and entrapment efficiency. Formulation LZL1 contained the lowest amount of phospholipid (100 mg) and cholesterol (25 mg), while LZL5 had the highest (250 mg of phospholipid and 60 mg of cholesterol). These gradients helped evaluate how lipid content influences vesicle stability, drug encapsulation, and release behavior. Increasing phosphatidylcholine typically enhances bilayer formation and drug encapsulation, whereas excessive cholesterol may lead to rigid vesicle membranes, affecting drug release.

In addition to lipid variation, sonication time and hydration volume were also evaluated. A fixed sonication time of 5 minutes was initially selected for all formulations to reduce particle size and achieve uniformity. Prolonged sonication times were avoided to prevent vesicle disruption and drug leakage. Similarly, a constant hydration volume of 10 mL PBS (pH 7.4) was maintained across all formulations to ensure consistent hydration of the thin lipid film and to facilitate reproducibility. Each formulation was subjected to a preliminary screening based on its particle size, zeta potential, and entrapment efficiency. LZL3 emerged as the optimal formulation, showing the smallest and most uniform particle size, high negative zeta potential, and maximum entrapment efficiency. This indicates a well-balanced lipid composition and vesicle stability suitable for sustained and targeted drug delivery applications. The optimization process thus enabled the selection of a lead formulation with ideal physicochemical and functional characteristics, suitable for further in vitro and in vivo evaluations

2.4. Characterization of Liposomes

2.4.1. Particle Size, Polydispersity Index (PDI), and Zeta Potential

The particle size, polydispersity index (PDI), and zeta potential of the prepared Linezolid-loaded liposomal formulations were critically evaluated to assess their physical stability, uniformity, and suitability for effective drug delivery. These parameters play a pivotal role in determining the in vivo behavior of liposomes, including their circulation time, biodistribution, and cellular uptake(Chaurasiya et al., 2022; Guimarães et al., 2021; Liu et al., 2022). Measurements were carried out using a Malvern Zetasizer Nano ZS90 (Malvern Instruments Ltd., UK), based on the principle of dynamic light scattering (DLS). Prior to analysis, a small volume of each liposomal formulation was appropriately diluted with double-distilled water to avoid multiple scattering effects and ensure optimal sample transparency for light detection. The average particle size was recorded in nanometers (nm) and reflected the hydrodynamic diameter of the liposomal vesicles in dispersion. Smaller and uniform particle sizes are desirable for enhancing drug absorption, reducing opsonization, and promoting deeper tissue penetration. A target size range below 200 nm was aimed for, as such nano-sized systems are considered ideal for passive targeting via the enhanced permeability and retention (EPR) effect.

The polydispersity index (PDI) served as an indicator of particle size distribution within the sample. Values below 0.3 denote a narrow and homogeneous distribution of vesicle sizes, essential for consistent therapeutic performance and physical stability. PDI values were directly obtained from the software output of the Zetasizer system (Chaurasiya et al., 2022; Guimarães et al., 2021; Liu et al., 2022).

The zeta potential, expressed in millivolts (mV), was determined by electrophoretic light scattering and provided insight into the surface charge of the liposomes. This electrokinetic parameter is vital for predicting colloidal stability; high absolute zeta potential values (either positive or negative) indicate strong repulsion between particles, thus preventing aggregation over time. In this study, a zeta potential of –20 mV or more negative was considered sufficient for achieving electrostatic stability in aqueous dispersion. Together, these measurements offered a comprehensive understanding of the liposomal formulations' stability profile and supported the selection of the most promising candidate for further

evaluation based on optimal size, uniformity, and surface charge (Chaurasiya et al., 2022; Guimarães et al., 2021; Liu et al., 2022).

2.4.2. Morphological Examination

The surface morphology and structural characteristics of the Linezolid-loaded liposomal formulations were assessed using Transmission Electron Microscopy (TEM), a high-resolution imaging technique capable of visualizing nanoscale structures. This analysis was performed to confirm the shape, size consistency, and integrity of the vesicles observed in dynamic light scattering (DLS) measurements. A freshly prepared liposomal dispersion was selected for imaging. A small drop of the sample was carefully deposited onto a carbon-coated copper grid using a micropipette. The grid was allowed to stand undisturbed for 1–2 minutes to facilitate the adsorption of liposomes onto the carbon film. Excess sample was gently removed with filter paper to avoid drying artifacts. To enhance image contrast and define the liposomal boundaries, the adhered liposomes were negatively stained with 1% phosphotungstic acid (PTA). The stain was applied by adding a small drop to the grid and allowing it to interact for 30 seconds. Excess stain was blotted off using filter paper, and the grid was left to air-dry at room temperature. The prepared grid was then mounted onto the sample holder and examined under a Transmission Electron Microscope operating at an accelerating voltage typically in the range of 80–120 kV. The images were captured digitally at different magnifications. TEM analysis revealed that the liposomes were predominantly spherical and uniformly dispersed, with smooth surfaces and well-defined bilayer structures. The particle sizes observed under TEM were in close agreement with those measured by DLS, supporting the accuracy and reliability of the size data. No significant aggregation or deformation was noted, indicating good formulation stability. This morphological analysis confirmed the successful formation of nanosized unilamellar liposomes with desirable structural features suitable for further drug delivery applications (Jash et al., 2021; Łukawski et al., 2020; Malik et al., 2017; Sheoran et al., 2019).

2.4.3. Entrapment Efficiency (EE%)

The entrapment efficiency (EE%) of the Linezolid-loaded liposomal formulations was determined to quantify the percentage of drug successfully incorporated within the lipid bilayers. This parameter is crucial in evaluating the formulation's drug-loading capacity and therapeutic viability, as higher entrapment typically translates to improved sustained-release performance and reduced dosing frequency. To assess EE%, a known volume (typically 2 mL) of each liposomal dispersion was subjected to ultracentrifugation at 15,000 rpm for 1 hour at 4°C using a high-speed refrigerated centrifuge. This step facilitated the sedimentation of liposomes, while the unentrapped (free) drug remained in the supernatant. After centrifugation, the clear supernatant was carefully collected without disturbing the pellet and was analyzed for free drug concentration. Quantification was carried out using UV-Visible spectrophotometry at 251 nm, the λmax for Linezolid, against a standard calibration curve prepared in phosphate-buffered saline (PBS, pH 7.4). The amount of encapsulated drug was calculated by subtracting the free drug content from the total drug initially added to the formulation. The entrapment efficiency (EE%) was then computed using the following formula(Jash et al., 2021; Łukawski et al., 2020; Malik et al., 2017; Sheoran et al., 2019):

$$EE\% = (\text{Total drug} - \text{Free drug}\text{Total drug}) \times 100$$

2.5. In Vitro Drug Release Study

The in vitro release profile of Linezolid from the liposomal formulations was evaluated using the dialysis bag diffusion method, a widely accepted technique for assessing the release kinetics of nanoscale drug delivery systems. This method effectively simulates drug diffusion through a semi-permeable membrane, mimicking biological barriers in vivo. For each formulation, 1 mL of the liposomal dispersion was accurately transferred into a pre-soaked dialysis membrane (molecular weight cut-off 12–14 kDa), which had been conditioned in distilled water for at least 12 hours to ensure flexibility and membrane integrity. The sealed membrane was then immersed into a beaker containing 100 mL of phosphate-buffered saline (PBS, pH 7.4), which served as the release medium. The experiment was conducted in a thermostatically controlled shaking water bath or magnetic stirrer maintained at 37 ± 0.5°C, mimicking physiological conditions. Continuous stirring at 100 rpm ensured uniform distribution of the released drug in the medium and prevented the formation of concentration gradients around the dialysis bag. At predetermined time intervals (0.5, 1, 2, 4, 6, 8, 10, and 12 hours), 2 mL aliquots were withdrawn from

the release medium and immediately replaced with an equal volume of fresh PBS to maintain sink conditions. The collected samples were filtered (if necessary) and analyzed using a UV-Visible spectrophotometer at 251 nm, the absorption maximum (λmax) of Linezolid, to quantify the amount of drug released. The percentage cumulative drug release was calculated and plotted against time to construct the release profile. All experiments were conducted in triplicate, and the results were presented as mean ± standard deviation. This method provided insight into the rate and extent of Linezolid release from various liposomal systems, allowing for comparative evaluation of release performance among formulations and aiding in the identification of the most effective sustained-release carrier (Jash et al., 2021; Łukawski et al., 2020; Malik et al., 2017; Sheoran et al., 2019).

2.6. Antibacterial Activity

The antibacterial efficacy of the developed Linezolid-loaded liposomal formulations (LZL1 to LZL5) was assessed against two representative bacterial strains: *Staphylococcus aureus* (Gram-positive) and *Escherichia coli* (Gram-negative). The evaluation was performed using the agar well diffusion method, a standard microbiological technique to qualitatively compare the antimicrobial potential of drug formulations. Fresh bacterial cultures were first standardized to 0.5 McFarland turbidity, corresponding to an approximate concentration of 1.5×10^8 CFU/mL, using a spectrophotometer. Mueller-Hinton agar (MHA) plates were prepared and uniformly seeded with the bacterial suspensions using a sterile cotton swab to ensure confluent growth across the plate surface. Sterile cork borers (6 mm in diameter) were used to punch wells into the agar, and each well was carefully loaded with 100 µL of the test samples (Li et al., 2022; Zhang et al., 2018):

- Liposomal formulation (LZL1 to LZL5),
- Free Linezolid solution (as a positive control), and
- Blank liposome (as a negative control).

The plates were incubated in an inverted position at 37°C for 24 hours under aseptic conditions. After incubation, the zones of inhibition (ZOI) around each well were measured using a calibrated scale in millimeters (mm). The diameter of the clear zone indicated the extent of antibacterial activity, with larger zones reflecting greater efficacy. All experiments were conducted in triplicate, and the results were expressed as mean ± standard deviation. The antibacterial activity of each liposomal formulation was compared with that of free Linezolid and the blank liposome to assess the enhancement (or retention) of drug activity post-encapsulation. This method helped determine whether liposomal encapsulation altered or enhanced the antimicrobial performance of Linezolid and which formulation offered the best combination of drug release and antibacterial potency.

2.7. Statistical Analysis

To ensure the reliability and reproducibility of the experimental results, all tests were conducted in triplicate, and the data obtained were analyzed and presented as mean ± standard deviation (SD). This statistical representation helped in understanding the variability within the data and provided a clearer picture of consistency among the replicates. For comparative analysis between different formulations and experimental groups, one-way analysis of variance (ANOVA) was employed. This test was used to determine whether significant differences existed among the means of the multiple formulation groups for each evaluated parameter—such as particle size, entrapment efficiency, drug release percentage, and antibacterial activity.

When the ANOVA results indicated significant differences ($p < 0.05$), a Tukey's post-hoc test was subsequently performed to identify which specific pairs of formulations differed significantly from one another. This step-wise approach minimized type I error and provided robust statistical validation of inter-formulation differences. All statistical analyses were performed using standard software tools such as GraphPad Prism or SPSS (version to be specified), and *p*-values less than 0.05 were considered to reflect statistical significance. This rigorous statistical evaluation was critical for validating the effectiveness of the optimized formulation (LZL3) and confirming its superiority over other variants.

3. RESULTS AND DISCUSSION

3.1 Physicochemical Characterization of Liposomes

The physicochemical parameters of the Linezolid-loaded liposomal formulations (LZL1–LZL5) were evaluated in terms of particle size, polydispersity index (PDI), zeta potential, and entrapment efficiency. As shown in Table 2, the particle size of the formulations ranged from 164.3 ± 2.3 nm (LZL3) to 212.4 ± 3.1 nm (LZL1). The decrease in particle size observed in LZL3 was likely due to the optimized lipid-to-cholesterol ratio and efficient sonication, which promoted the formation of smaller unilamellar vesicles. The PDI values of all formulations were below 0.3, indicating a narrow size distribution and uniformity of vesicles. Notably, LZL3 exhibited the lowest PDI (0.247), reinforcing its superior homogeneity. Zeta potential values ranged from –23.5 to –27.6 mV. The relatively high negative charge of LZL3 (–27.6 ± 0.9 mV) suggested better electrostatic stability due to stronger repulsion between particles, minimizing aggregation. Entrapment efficiency (EE%) was significantly influenced by the lipid and cholesterol concentrations. LZL3 exhibited the highest entrapment efficiency (81.56 ± 2.5%), likely due to the optimal phosphatidylcholine content (200 mg) that enhanced bilayer formation and drug encapsulation. In contrast, LZL1 showed the lowest EE% (65.42 ± 2.1%) due to its lower lipid content, which provided less encapsulating matrix for the drug. Overall, LZL3 displayed superior physicochemical characteristics, including small and uniform particle size, high zeta potential, and excellent entrapment efficiency, making it a robust formulation for further pharmacological evaluation.

Table 2: Physicochemical Characterization of Linezolid-Loaded Liposomes

Formulation Code	Particle Size (nm)	PDI	Zeta Potential (mV)	Entrapment Efficiency (%)
LZL1	212.4 ± 3.1	0.312	–23.5 ± 1.2	65.42 ± 2.1
LZL2	186.7 ± 2.6	0.289	–25.1 ± 1.0	72.88 ± 1.8
LZL3	164.3 ± 2.3	0.247	–27.6 ± 0.9	81.56 ± 2.5
LZL4	172.5 ± 2.1	0.255	–26.8 ± 1.1	78.13 ± 1.9
LZL5	198.9 ± 3.5	0.291	–24.4 ± 1.3	70.27 ± 2.3

Figure 1. Particle Size (nm)

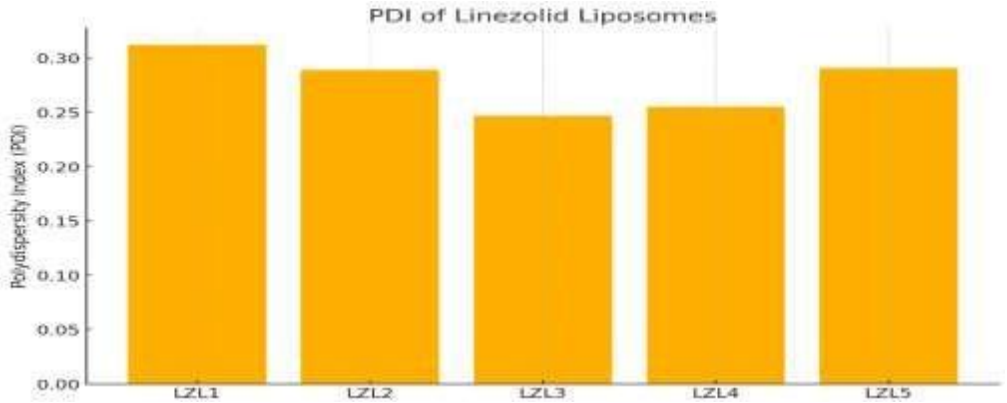

Figure 2. PDI

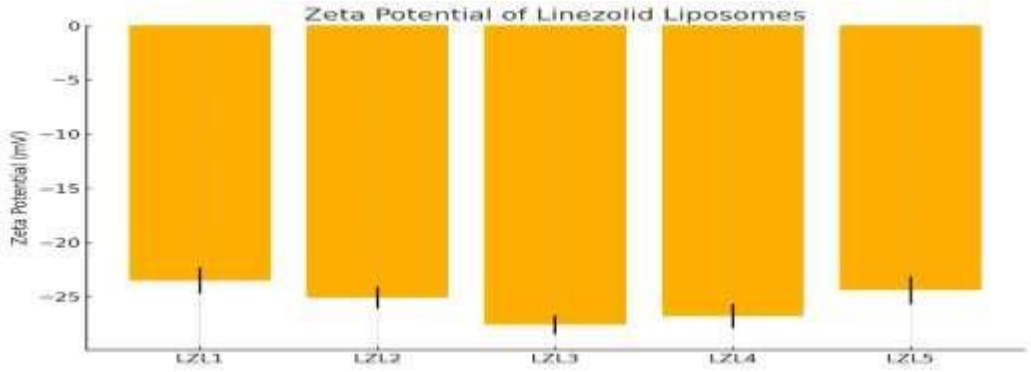

Figure 3. Zeta Potential (mV)

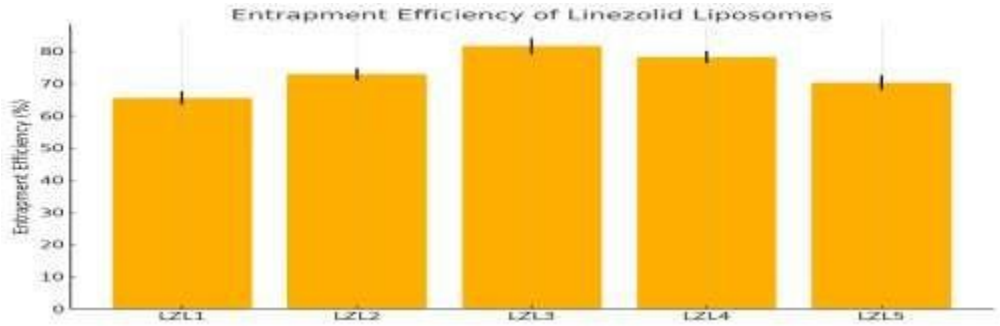

Figure 4. Entrapment Efficiency (%)

3.2 Morphiological study usinh TEM

The surface morphology and structural characteristics of the Linezolid-loaded liposomal formulations were examined using Transmission Electron Microscopy (TEM). This high-resolution imaging technique allowed direct visualization of vesicle shape, surface texture, and overall uniformity, complementing the particle size data obtained through dynamic light scattering (DLS). The representative TEM micrograph revealed that the liposomes were predominantly spherical in shape with smooth and well-defined boundaries, confirming successful formation of nanosized vesicles. The images showed discrete and uniformly distributed particles, indicating good physical stability without signs of aggregation or fusion. These observations are consistent with the low polydispersity index (PDI) values recorded for the formulations, especially LZL3, which exhibited the most uniform morphology. Furthermore, the average particle size observed under TEM closely matched the DLS data, falling within the 150–220 nm range. The liposomal structures appeared unilamellar, and the absence of irregularities or structural collapse confirmed the integrity of the vesicles post-sonication. Overall, the TEM analysis validated the successful development of structurally stable liposomes with uniform nanoscale characteristics, suitable for enhancing drug delivery performance in targeted antibacterial therapy.

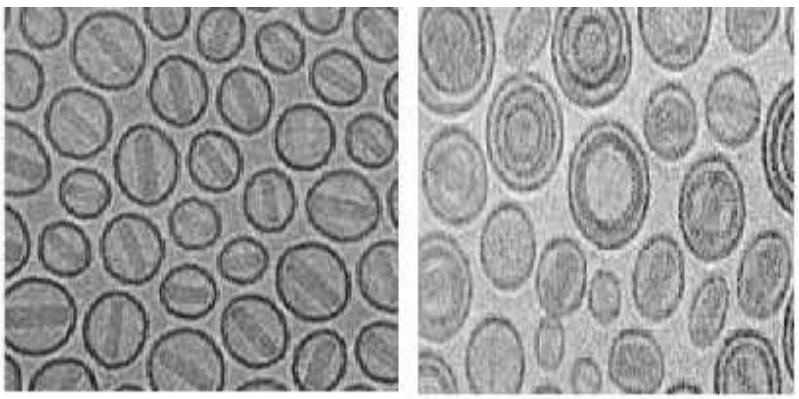

Figure 5. Representative TEM photographs of Linezolid-Loaded Liposomes

3.3 In Vitro Drug Release Profile

The in vitro drug release study was conducted over a 12-hour period to assess the release characteristics of Linezolid from the five liposomal formulations (LZL1 to LZL5). The results revealed a biphasic release pattern characterized by an initial burst release followed by a sustained release phase. Among all formulations, LZL3 exhibited the highest cumulative drug release, reaching 88.56 ± 1.89% at 12 hours, suggesting optimal formulation characteristics such as ideal lipid-to-cholesterol ratio and vesicle integrity. Formulations LZL2 and LZL4 followed closely, releasing 71.84 ± 1.98% and 81.13 ± 2.08% of Linezolid respectively at 12 hours. LZL1 and LZL5 demonstrated relatively slower release, possibly due to lower phospholipid content (in LZL1) or excessive cholesterol (in LZL5), which may have contributed to tighter bilayer packing and hindered drug diffusion. The initial release (0.5 to 2 hours) showed that LZL3 also released a higher percentage of Linezolid during the burst phase, indicating better surface availability of the drug. The sustained release behavior observed after 2 hours can be attributed to the gradual diffusion of the drug entrapped within the lipid bilayers. These findings suggest that LZL3 offers a balanced and controlled drug release, making it a promising candidate for targeted antibacterial therapy requiring sustained delivery.

Table 3: In Vitro Drug Release Profile (% Cumulative Drug Release) from 0.5 to 12 Hours

Time (h)	LZL1	LZL2	LZL3	LZL4	LZL5
0.5	12.34 ± 0.85	14.98 ± 0.77	18.42 ± 0.69	16.11 ± 0.84	13.25 ± 0.66
1	21.48 ± 1.02	26.11 ± 0.94	31.57 ± 0.82	28.37 ± 1.06	23.49 ± 0.91
2	31.65 ± 1.28	38.76 ± 1.23	47.64 ± 1.05	42.93 ± 1.18	34.71 ± 1.07
4	44.72 ± 1.37	53.81 ± 1.30	65.18 ± 1.23	59.26 ± 1.41	48.66 ± 1.22
6	52.98 ± 1.55	61.94 ± 1.46	74.21 ± 1.36	68.42 ± 1.53	56.77 ± 1.31
8	58.73 ± 1.62	67.88 ± 1.57	82.34 ± 1.42	75.94 ± 1.66	62.85 ± 1.39
10	61.25 ± 1.71	70.22 ± 1.65	86.41 ± 1.58	79.37 ± 1.72	66.49 ± 1.46
12	64.23 ± 1.70	71.84 ± 1.98	88.56 ± 1.89	81.13 ± 2.08	68.45 ± 1.59

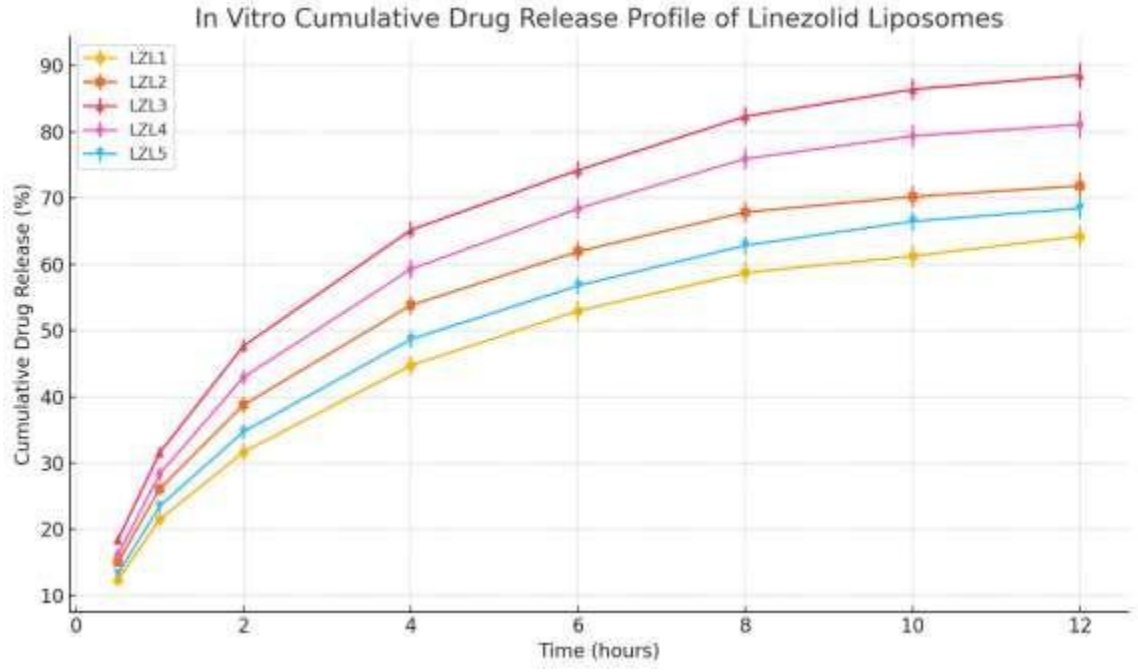

Figure 6. In Vitro Drug Release Profile (% Cumulative Drug Release) from 0.5 to 12 Hours

3.3 Antibacterial Activity

The antibacterial efficacy of the Linezolid-loaded liposomal formulations was evaluated against *Staphylococcus aureus* and *Escherichia coli* using the agar well diffusion method. The results, summarized in Table 4, demonstrated that all drug-loaded formulations exhibited inhibitory activity against both

bacterial strains, while the blank liposome showed no zone of inhibition, confirming the absence of intrinsic antibacterial properties in the lipid components. Among the formulations, LZL3 showed the highest antibacterial activity, with a zone of inhibition of 20.4 ± 0.7 mm against *S. aureus* and 18.9 ± 0.6 mm against *E. coli*. This superior activity may be attributed to its smaller particle size, higher drug entrapment, and enhanced release profile, which collectively facilitated better interaction with the bacterial cell membranes and more effective drug delivery at the site of infection. LZL2 and LZL4 also showed moderate antibacterial effects, correlating with their relatively high entrapment and release profiles. In contrast, LZL1 and LZL5 exhibited lower zones of inhibition, likely due to suboptimal drug release and entrapment. The free Linezolid solution produced slightly larger inhibition zones (22.1 ± 0.6 mm for *S. aureus*, 21.5 ± 0.7 mm for *E. coli*), indicating immediate and unrestricted drug diffusion. However, liposomal formulations, especially LZL3, offer the advantage of sustained and targeted delivery, which may be more beneficial in prolonged infections and localized therapy. These findings validate the potential of liposomal encapsulation in enhancing the antibacterial activity of Linezolid through controlled release and targeted delivery.

Table 4: Antibacterial Activity (Zone of Inhibition in mm)

Formulation Code	**Zone of Inhibition in mm**	
	S. aureus	***E. coli***
LZL1	14.2 ± 0.5	13.5 ± 0.4
LZL2	16.8 ± 0.6	15.6 ± 0.5
LZL3	20.4 ± 0.7	18.9 ± 0.6
LZL4	18.7 ± 0.5	17.4 ± 0.4
LZL5	15.9 ± 0.4	14.8 ± 0.5
Free Linezolid (Std)	22.1 ± 0.6	21.5 ± 0.7
Blank Liposome	0.0	0.0

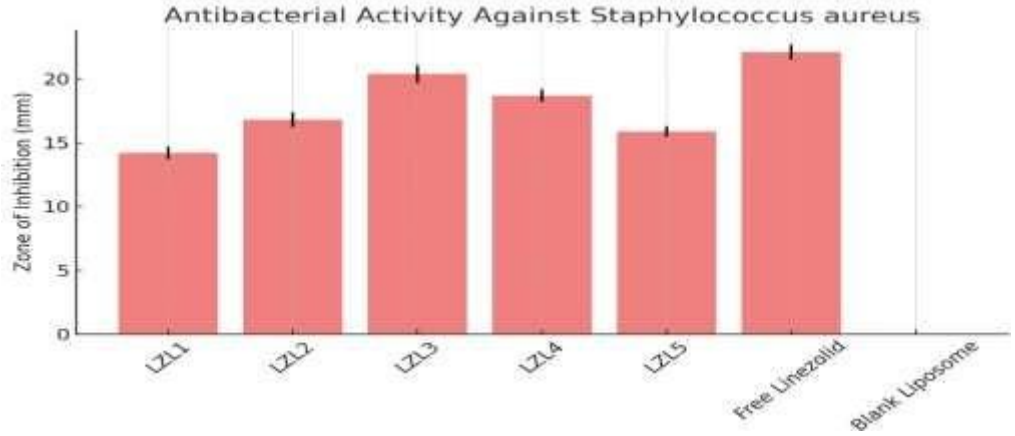

Figure 7. Antibacterial Activity (Zone of Inhibition in mm) agaisnst *S. aureus*

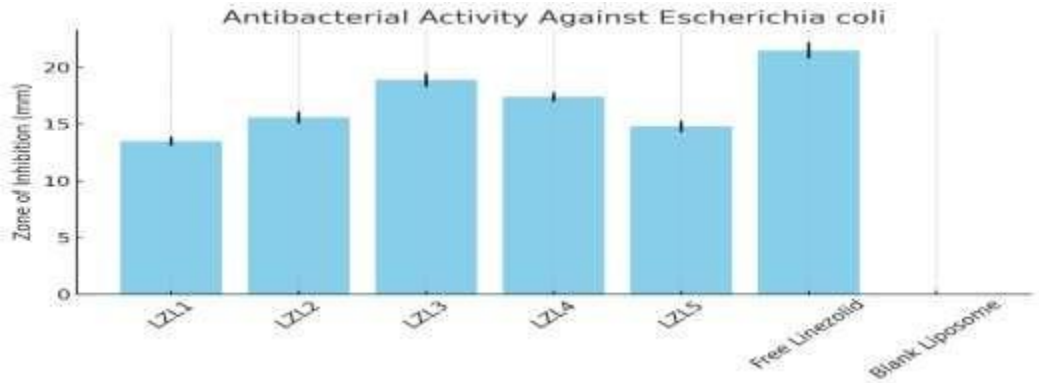

Figure 8. Antibacterial Activity (Zone of Inhibition in mm) agaisnst *E. coli*

4. CONCLUSIONS

This study successfully developed and characterized Linezolid-loaded liposomal formulations with the goal of enhancing antibacterial efficacy through targeted and sustained drug delivery. The liposomes were formulated using the thin-film hydration technique followed by probe sonication, and five different compositions were systematically optimized based on lipid and cholesterol concentrations. Among all tested formulations, LZL3 emerged as the most promising candidate, demonstrating superior physicochemical properties, including small particle size (164.3 ± 2.3 nm), low PDI (0.247), and a highly negative zeta potential (–27.6 ± 0.9 mV), indicating good colloidal stability. The entrapment efficiency of 81.56 ± 2.5% confirmed efficient drug loading. TEM imaging confirmed the spherical morphology and nanoscale structure of the vesicles, further validating successful liposome formation. In vitro release studies highlighted the controlled and sustained release behavior of LZL3, with nearly 89% of Linezolid released over 12 hours. This sustained release could offer potential advantages in reducing dosing frequency and improving patient compliance. Furthermore, antibacterial activity studies against S. aureus and E. coli showed that LZL3 exhibited significant inhibition zones, closely matching that of the free drug and outperforming other formulations. Overall, the study demonstrated that liposomal encapsulation of Linezolid not only preserves but may enhance its antibacterial activity. The optimized formulation (LZL3) holds considerable potential for clinical translation, particularly in the treatment of resistant bacterial infections where sustained delivery is crucial. Liposomes offer a versatile and biocompatible drug delivery system that can reduce systemic toxicity and improve site-specific drug accumulation. Future investigations may involve in vivo pharmacokinetics and efficacy studies to further establish the therapeutic benefits of the developed liposomal formulation for targeted antibacterial therapy.

REFERENCES

1. Ahmed, S., Ahmed, M. Z., Rafique, S., Almasoudi, S. E., Shah, M., Jalil, N. A. C., & Ojha, S. C. (2023). Recent Approaches for Downplaying Antibiotic Resistance: Molecular Mechanisms. *Biomed Res Int*, *2023*, 5250040. https://doi.org/10.1155/2023/5250040
2. Ahsan, A., Thomas, N., Barnes, T. J., Subramaniam, S., Loh, T. C., Joyce, P., & Prestidge, C. A. (2024). Lipid Nanocarriers-Enabled Delivery of Antibiotics and Antimicrobial Adjuvants to Overcome Bacterial Biofilms. *Pharmaceutics*, *16*(3). https://doi.org/10.3390/pharmaceutics16030396
3. Brinkac, L., Voorhies, A., Gomez, A., & Nelson, K. E. (2017). The Threat of Antimicrobial Resistance on the Human Microbiome. *Microb Ecol*, *74*(4), 1001-1008. https://doi.org/10.1007/s00248-017-0985-z
4. Catalano, A., Iacopetta, D., Ceramella, J., Scumaci, D., Giuzio, F., Saturnino, C., Aquaro, S., Rosano, C., & Sinicropi, M. S. (2022). Multidrug Resistance (MDR): A Widespread Phenomenon in Pharmacological Therapies. *Molecules*, *27*(3). https://doi.org/10.3390/molecules27030616
5. Chahar, K., Sharma, Y., Patel, P., Asati, V., & Das Kurmi, B. (2023). A Mini-review on Recent Strategies and Applications of Nanomedicines to Combat Antimicrobial Resistance. *Curr Drug Metab*, *24*(6), 406-421. https://doi.org/10.2174/1389200224666230731093319
6. Chaurasiya, A., Gorajiya, A., Panchal, K., Katke, S., & Singh, A. K. (2022). A review on multivesicular liposomes for pharmaceutical applications: preparation, characterization, and translational challenges. *Drug Deliv Transl Res*, *12*(7), 1569-1587. https://doi.org/10.1007/s13346-021-01060-y
7. Conradie, F., Bagdasaryan, T. R., Borisov, S., Howell, P., Mikiashvili, L., Ngubane, N., Samoilova, A., Skornykova, S., Tudor, E., Variava, E., Yablonskiy, P., Everitt, D., Wills, G. H., Sun, E., Olugbosi, M., Egizi, E., Li, M., Holsta, A., Timm, J., . . . Spigelman, M. (2022). Bedaquiline-Pretomanid-Linezolid Regimens for Drug-Resistant Tuberculosis. *N Engl J Med*, *387*(9), 810-823. https://doi.org/10.1056/NEJMoa2119430
8. Crass, R. L., Cojutti, P. G., Pai, M. P., & Pea, F. (2019). Reappraisal of Linezolid Dosing in Renal Impairment To Improve Safety. *Antimicrob Agents Chemother*, *63*(8). https://doi.org/10.1128/aac.00605-19
9. Franco-Gonzalez, J. F., Matamoros-Recio, A., Torres-Mozas, A., Rodrigo-Lacave, B., & Martin-Santamaria, S. (2022). Lipid-A-dependent and cholesterol-dependent dynamics properties of liposomes from gram-negative bacteria in ESKAPE. *Sci Rep*, *12*(1), 19474. https://doi.org/10.1038/s41598-022-22886-7
10. Guimarães, D., Cavaco-Paulo, A., & Nogueira, E. (2021). Design of liposomes as drug delivery system for therapeutic applications. *Int J Pharm*, *601*, 120571. https://doi.org/10.1016/j.ijpharm.2021.120571
11. Hashemian, S. M. R., Farhadi, T., & Ganjparvar, M. (2018). Linezolid: a review of its properties, function, and use in critical care. *Drug Des Devel Ther*, *12*, 1759-1767. https://doi.org/10.2147/dddt.S164515
12. Jash, A., Ubeyitogullari, A., & Rizvi, S. S. H. (2021). Liposomes for oral delivery of protein and peptide-based therapeutics: challenges, formulation strategies, and advances. *J Mater Chem B*, *9*(24), 4773-4792. https://doi.org/10.1039/d1tb00126d
13. Li, Z., Zhao, L., Bian, Y., Li, Y., Qu, J., & Song, F. (2022). The Antibacterial Activity of Quinazoline and Quinazolinone Hybrids. *Curr Top Med Chem*, *22*(12), 1035-1044. https://doi.org/10.2174/1568026622666220307144015

14. Liu, G., Hou, S., Tong, P., & Li, J. (2022). Liposomes: Preparation, Characteristics, and Application Strategies in Analytical Chemistry. *Crit Rev Anal Chem*, *52*(2), 392-412. https://doi.org/10.1080/10408347.2020.1805293
15. Łukawski, M., Dałek, P., Borowik, T., Foryś´, A., Langner, M., Witkiewicz, W., & Przybyło, M. (2020). New oral liposomal vitamin C formulation: properties and bioavailability. *J Liposome Res*, *30*(3), 227-234. https://doi.org/10.1080/08982104.2019.1630642
16. Malik, D. J., Sokolov, I. J., Vinner, G. K., Mancuso, F., Cinquerrui, S., Vladisavljevic, G. T., Clokie, M. R. J., Garton, N. J., Stapley, A. G. F., & Kirpichnikova, A. (2017). Formulation, stabilisation and encapsulation of bacteriophage for phage therapy. *Adv Colloid Interface Sci*, *249*, 100-133. https://doi.org/10.1016/j.cis.2017.05.014
17. Marchianò, V., Matos, M., Serrano-Pertierra, E., Gutiérrez, G., & Blanco-López, M. C. (2020). Vesicles as antibiotic carrier: State of art. *Int J Pharm*, *585*, 119478. https://doi.org/10.1016/j.ijpharm.2020.119478
18. Mehrotra, T., Konar, D., Pragasam, A. K., Kumar, S., Jana, P., Babele, P., Paul, D., Purohit, A., Tanwar, S., Bakshi, S., Das, S., Verma, J., Talukdar, D., Narendrakumar, L., Kothidar, A., Karmakar, S. P., Chaudhuri, S., Pal, S., Jain, K., . . . Das, B. (2023). Antimicrobial resistance heterogeneity among multidrug-resistant Gram-negative pathogens: Phenotypic, genotypic, and proteomic analysis. *Proc Natl Acad Sci U S A*, *120*(33), e2305465120. https://doi.org/10.1073/pnas.2305465120
19. Miller, H. V., Cao, A. A., McClelland, C. M., & Lee, M. S. (2023). Linezolid optic neuropathy. *Curr Opin Ophthalmol*, *34*(6), 481-486. https://doi.org/10.1097/icu.0000000000000995
20. Qiao, M., Ying, G. G., Singer, A. C., & Zhu, Y. G. (2018). Review of antibiotic resistance in China and its environment. *Environ Int*, *110*, 160-172. https://doi.org/10.1016/j.envint.2017.10.016
21. Rao, G. G., Konicki, R., Cattaneo, D., Alffenaar, J. W., Marriott, D. J. E., & Neely, M. (2020). Therapeutic Drug Monitoring Can Improve Linezolid Dosing Regimens in Current Clinical Practice: A Review of Linezolid Pharmacokinetics and Pharmacodynamics. *Ther Drug Monit*, *42*(1), 83-92. https://doi.org/10.1097/ftd.0000000000000710
22. Sheoran, R., Khokra, S. L., Chawla, V., & Dureja, H. (2019). Recent Patents, Formulation Techniques, Classification and Characterization of Liposomes. *Recent Pat Nanotechnol*, *13*(1), 17-27. https://doi.org/10.2174/1872210513666181127110413
23. Vairo, C., Villar Vidal, M., Maria Hernandez, R., Igartua, M., & Villullas, S. (2023). Colistin- and amikacin-loaded lipid-based drug delivery systems for resistant gram-negative lung and wound bacterial infections. *Int J Pharm*, *635*, 122739. https://doi.org/10.1016/j.ijpharm.2023.122739
24. Zahedi Bialvaei, A., Rahbar, M., Yousefi, M., Asgharzadeh, M., & Samadi Kafil, H. (2017). Linezolid: a promising option in the treatment of Gram-positives. *J Antimicrob Chemother*, *72*(2), 354-364. https://doi.org/10.1093/jac/dkw450
25. Zhang, G. F., Liu, X., Zhang, S., Pan, B., & Liu, M. L. (2018). Ciprofloxacin derivatives and their antibacterial activities. *Eur J Med Chem*, *146*, 599-612. https://doi.org/10.1016/j.ejmech.2018.01.078