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ADVANTAGES OF USING LIPOSOME-ENCAPSULATED ANTIBIOTICS TO FIGHT INFECTIONS CAUSED BY ENTEROBACTERIA

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ABSTRACT:

Introduction: Treating illnesses caused by enterobacteria is increasingly challenging due to the ineffectiveness of antibiotics in combating pathogens or promoting reticuloendothelial system cell phagocytosis, especially for bacteria that colonize and grow inside phagocytic cells. Certain antibiotics are unable to penetrate cells or are limited by the plasma membrane. Consequently, there is a scientific effort to develop new therapeutic strategies to overcome these challenges. Lipid nanocarriers, such as liposomes, can encapsulate antibiotics to enhance their delivery, targeting, and efficacy. Liposomes aim to improve the specificity of release, concentration of compounds at the target site, maintenance of drug plasma concentration, and preservation of active components. This review aims to outline the key characteristics of liposomes and emphasize the benefits of using these lipid vesicles to deliver antibiotics against illnesses caused by enterobacteria.

Methodology: A literature review was conducted by searching national and international electronic databases for articles published between 2012 and 2023. Articles were selected based on the following descriptors: bacterial resistance, nanotechnology, gram-negative bacteria, and antibiotics.

Findings: Antibacterial activity has been demonstrated for cationic and stealth liposomes, primarily composed of cholesterol, PEG, phosphatidylcholine, and carboxymethyl chitosan encapsulating drugs such as amoxicillin, ciprofloxacin, cloxacillin, vancomycin, azithromycin, amoxicillin, cefepime, gentamicin, and cefotaxime. Liposomes containing polymyxin B, azithromycin, chloramphenicol, and gentamicin have shown improved antibiofilm efficacy against enterobacteria compared to non-encapsulated pharmaceuticals.
Conclusion: The findings highlight the significant therapeutic potential of liposomes in the management of enterobacteria-related illnesses.

KEYWORDS: Bacterial resistance; Gram-negative bacteria; Nanotechnology; Antimicrobials.

INTRODUCTION:

Current drug therapy for infections caused by enterobacteria is a challenge in the clinic, mainly because some bacteria are drug-tolerant and persist due to bacterial resistance. Resistance to antimicrobial agents is a natural condition accelerated by several factors, including the irrational use of antimicrobials that causes major complications in the fight against these pathogens. This resistance can occur through genetic mutations or bacterial recombination methods, making drug therapy difficult (Gkartziou & Antimisiaris, 2024; Pinilla, Lopes, & Brandelli, 2023).

In addition to acquiring resistance due to genetic changes, bacteria are also able to form biofilms, which are considered a survival mechanism of bacteria as well as hindering the interaction between the drug and the bacteria. Several European hospitals have reported outbreaks since the 1990s, confirming the epidemiological concern towards these multidrug-resistant (MDR) pathogens. The World Health Organization (WHO) published in 2017 a catalogue of 12 families of MDR bacteria that pose a threat to human health. The list was created to promote research and development of new antibiotics to treat these bacteria (Ahsan et al., 2024; Panthi, Fairfull-Smith, & Islam, 2024).

Enterobacteriaceae, which includes bacteria like E. coli, Klebsiella sp., Serratia sp., and Proteus sp., are among the germs that can cause serious, recurring, and deadly infections. Enterobacteriaceae is one of the major categories of pathogenic bacteria that either develop or exhibit resistance. A vast class of Gram-negative bacteria known as Enterobacteriaceae is found naturally in the gastrointestinal tracts of many different kinds of animals. However, this family also includes certain dangerous bacteria, including Salmonella enterica, Escherichia coli, Shigella spp., and Klebsiella pneumoniae. There are many challenges in treating Enterobacteriaceae infections, such as limited bioavailability, neurotoxicity, nephrotoxicity, and low drug penetration into infected cells, which lowers the effectiveness of the treatments and patient adherence to therapy (Plotniece et al., 2023; Pylypenko, Grigoryeva, & Krasnopolsky, 2023).

METHODOLOGY:

The national and international electronic databases Medline, SCIELO, Scholar Google (Google Scholar), PubMed, and Virtual Health Library (BVS) were searched for this bibliographic review, which takes a qualitative and exploratory approach. Only English-language articles published between 2012 and 2023 were chosen for the review based on the following criteria: Antimicrobials, nanotechnologies, bacterial resistance, and gram-negative bacteria (Bolsan et al., 2024; Yao et al., 2023).

RESULTS AND DISCUSSION:

PROBLEMS OF INFECTIONS CAUSED BY ENTEROBACTERIA:

Enterobacteria are a large family of Gram-negative bacteria, non-spore-forming facultative anaerobic bacilli, most of which can grow at temperatures between 25 and 37 °C. There are about 53 genera of Enterobacteriaceae, of which more than 170 species have been named. This group is widely present in nature, such as soil, water, plants, and the gastrointestinal microbiota of humans and animals, where

Escherichia coli is the most abundant species of the family. Among the 53 genera of Enterobacteriaceae, about 26 genera have been associated with infections in humans (Saxena et al., 2023; Scoffone, Barbieri, Irudal, Trespidi, & Buroni, 2024).

The main pathogens of great epidemiological importance that cause global morbidity and mortality and the onset of MDR are Salmonella, Escherichia, Shigella, and Yersinia. The main infections associated with enterobacteria are bacteremia, lower respiratory tract infections, skin infections, urinary tract infections (UTI), intra-abdominal infections, ophthalmic infections, and other infections. When MDR bacteria infect and cause disease in an individual, they pose serious challenges for treatment, being considered the second leading cause of mortality worldwide (Manicum et al., 2023; Roy, Hasan, & Guo, 2023).

The resistance of these microorganisms is accelerated by the indiscriminate use of antibiotics, together with the release of large quantities of antibiotics into the environment through hospital wastewater. These residues are not treated and can impact the aquatic bacterial niche and cause genetic changes, resulting in bacterial resistance to drugs. These pathogens are capable of acquiring different resistance mechanisms through different genes that modify the outer membrane of the bacteria, alter the internal cell structure, modify membrane transporters such as porins and produce efflux pumps, produce enzymes such as extended-spectrum β-lactamases (ESBLs) and carbapenemases, such as Klebsiella oxytoca, which possesses K1 β-lactamase enzymes that cause resistance to cephalosporins and aztreonam; Proteus vulgaris which can resist antibiotic treatment due to the expression of chromosomal β-lactamases, among others (Mohanty, Suar, & Panda, 2023; D.-Y. Wang, 2023).

The genes encoding these enzymes can also be found in the chromosomes and plasmids of E. coli that produce chromosomal β-lactamases that help in antibiotic resistance. Bacterial resistance can be transferred between species through some mobile genes that can spread mainly through international travellers who are at risk of contracting Enterobacteriaceae variants and, consequently, contracting infections caused by these bacteria. In addition to antibiotic resistance, microorganisms can live in biofilms, including Enterobacteriaceae. Biofilms are defined as a structured community of microorganisms surrounded by a self-produced matrix of extracellular polymeric substances that adhere to inert or living surfaces, thus forming a microbial survival mechanism (Johnson, Young, Gordon, & Preuss, 2023; Khambhati et al., 2023).

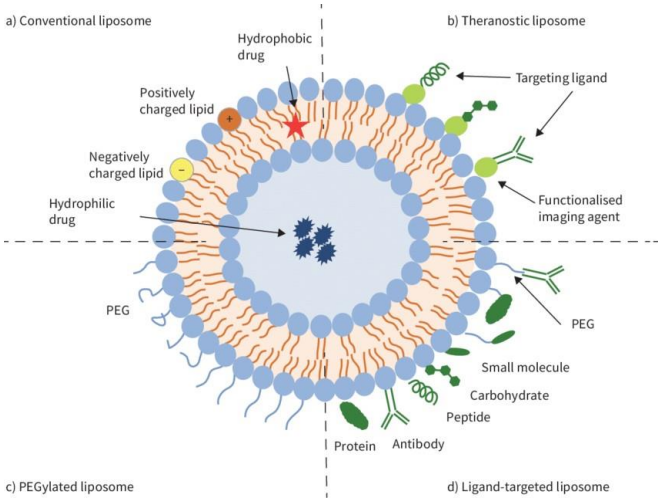
The matrix is made up of the aggregation of polysaccharides, proteins, and DNA, which favours an ecological niche, creating a compartment that allows survival to the stress of the local microenvironment and channels the evolution, community cooperation, spread, and recrudescence of new infections. The matrix also acts as a protective barrier that prevents drug interactions, ensuring microbiological survival. Within the biofilm, there can be a single species of bacteria or a consortium of multispecies microbial. Recent studies show the ability of Enterobacteriaceae to live in biofilms, such as the study conducted by Ramos-Vivas et al., which demonstrated biofilms of Escherichia coli, Klebsiella pneumoniae and Enterobacter spp. in samples from patients who underwent solid organ transplantation such as kidney, liver and pancreas (Allemailem, 2024; Y. Li, Li, Duan, Yang, & Ye, 2024).

In this study, 39 strains were isolated before transplantation and 179 strains after transplantation. Of these strains, many lived in biofilms, about 73% of K. pneumoniae, 16% of E. coli, and 4% of Enterobacter spp. Nosocomial infections caused by these MDR pathogens represent a global problem, becoming a major cause of death in recent years. Therefore, research groups are looking for effective therapeutic strategies for the treatment of infections caused by these pathogens, which could be based on the use of pharmaceutical nanotechnology through the use of liposomes (Fowoyo, 2024; Torres Di Bello, Narváez, Groot de Restrepo, & Vives, 2023).

LIPOSOMES AND THEIR ADVANTAGES FOR DRUG ENCAPSULATION:

Liposomes can be defined as lipid vesicles with an internal aqueous compartment capable of encapsulating hydrophilic and hydrophobic drugs. Liposomes are made of phospholipids and cholesterol, where the most used lipids contain phosphatidylcholine, phosphatidylserine, phosphatidylglycerol, and other natural and synthetic phospholipids and have vesicle diameters ranging from 20 to 5000 nm. Thanks to this composition, liposomes are biocompatible and biodegradable. Liposomes can have a single layer, called unilamellar, or multiple layers, called multilamellar. They have an amphiphilic nature due to two compartments: a hydrophilic/aqueous one located inside the liposome and a lipophilic one located between its membranes. In addition, the external membrane of the liposome can be loaded depending on the purpose for which the liposome is prepared and to prevent the aggregation of the vesicles, increasing their stability (Figure 1) (Bose, Sarkar, & Jo, 2024; Khater et al., 2023).

Figure 1. Structural characteristics of liposomes: conventional, stealth, site-specific, and pH sensitive.



The main advantages of using liposomes in therapy, including antimicrobial therapy, are the possibility of intracellular drug targeting, increased drug efficacy, and reduced toxicity. Liposomes also can create a protective environment for drugs, ensuring stability and plasma retention of the antibiotic for better efficacy in the treatment of infections. They can also improve the bioavailability and biodistribution of drugs in vivo, as well as preserving the pharmacokinetic and pharmacodynamic properties of the encapsulated drugs (Hajibonabi et al., 2023; B. Wang et al., 2024).

The first generation of liposomes, known as conventional liposomes, are formed by a biocompatible and biodegradable lipid bilayer, which encapsulates many therapeutic agents. However, when administered in vivo, they are rapidly captured in the bloodstream, taken to the reticuloendothelial system (RES) or mononuclear phagocytic system, which metabolizes liposomes, thereby reducing their concentration in the bloodstream and accumulates them in organs such as the liver, spleen, and bone marrow. Due to the lipid compartment of liposomes, how these vesicles interact with phagosomes are fusion interactions (Liu et al., 2024; Mehmood Khan et al., 2023).

Where the lipid layer and the cell plasma membrane mix and diffuse, thus allowing agents to be internalized in their aqueous or lipophilic core are released directly into the intracellular environment of target cells; lipid exchange between the liposomal vesicle and the host cell, given by lipid exchange proteins present on the cell surface; specific or non-specific adsorption of the liposome to the cell membrane which occurs in the absence of fusion between the liposomal layer and the cell membrane, resulting from attractive forces (electrodynamic interactions, van de Waals, hydrophobic insertion or hydrogen bonds) which exert repulsive forces, such as electrostatic interactions, steric, protrusion,

and adsorption of lipid vesicles on the phagosome membrane (Brandelli, Lopes, & Pinilla, 2023; Yan & Kim, 2024).

Liposomes can also be destabilized by cell membrane components when adsorbed on the surface, releasing their internal material into the cytoplasm via micropinocytosis or interaction by endocytosis, where cells of the mononuclear phagocytic system engulf liposomes with processes that form endosomes, where liposomes fuse to form a phagosome and lysosomal enzymes degrade the vesicle's phospholipids and release its substrate into the cytoplasm. After a few years, researchers succeeded in developing second-generation liposomes, also called stealth liposomes. These liposomes can increase the circulation time of liposomes in the bloodstream without being phagocytosed and metabolized by the SRE, thanks to the inclusion of polymers such as polyethene glycol (PEG) on the liposomal surface (Caselli, Rodrigues, Franco, & Malmsten, 2023; Paudel et al., 2024).

PEG chains can be incorporated into the liposome surface during preparation or after its formation by post-insertion. These chains can decrease hydrophobicity and immunogenicity and increase stability by preventing the opsonization of phagocytes and their removal from the bloodstream. The third generation of liposomes developed are site-specific liposomes. These nanocarriers can direct encapsulated drugs to specific locations, such as tumour tissues and microorganism-infected tissues, based on the attachment of ligands to the liposome surface, such as antibodies, proteins, peptides, and aptamers, which bind to specific components present at the desired location. This surface functionalization induces changes in drug delivery to the target cell, which can stimulate endocytosis and enhance the response after binding to the specific cellular receptor (Pushpalatha et al., 2024; Yang, Zhu, Xu, & Sun, 2023).

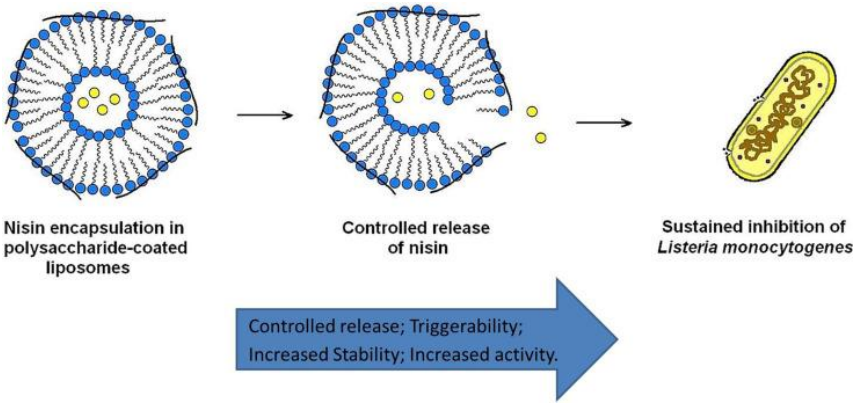
In addition to these liposomes mentioned above, other types have also been studied, such as pH-sensitive liposomes for disintegration in acidic media and thermosensitive liposomes for heat delivery. pH-sensitive liposomes are stimuli-sensitive nanocarriers that are pH-sensitive. These liposomes can, without lipid-polymer conjugates such as PEG-HZ-PE, provide a membrane with a long circulation function, thus allowing the liposome to find an acidic or basic pH environment for proper release and penetration. Due to their fusogenic potential, they are destabilized in the endosomal stage, which prevents drug degradation and stimulates the release of the drug into the cytoplasm of the cell. This allows them to carry gene fragments and drugs into the cytoplasm through the endocytic pathway (Drago et al., 2024; Tripathi, Singh, Trivedi, & Ranade, 2023).

Phase transition temperature (Tm) is the temperature at which the lipids that make up thermosensitive liposomes (LTS) experience phase transitions when exposed to high temperatures. LTS are carriers that are sensitive to hyperthermia stimuli. Tm is the point at which the lipids' physical state shifts from a gel phase to a permeable liquid phase, forming barriers that control the drug's release via membrane permeability. The temperature increase over the lipid mixture's Tm may enhance the permeability of the lipid bilayer, which in turn may affect the release of the medication enclosed in LTS. The lipid components of LTS include cholesterol, phosphatidylcholine, PEG, DSPC, and DPPC. One benefit of LTS is the ability to conjugate ligands specific to targets, which increases the drug's retention in key locations and enhances its therapeutic utility (Rajangam & Narasimhan, 2024; Zhang, Sharma, Tom, Liao, & Wu, 2023).

ENCAPSULATION OF ANTIBIOTICS IN LIPOSOMES FOR THE TREATMENT OF INFECTIONS CAUSED BY ENTEROBACTERIA:

Liposomes can act on bacterial cells through adhesion to the cytoplasmic membrane and the cell wall, penetrating them and, consequently, influencing the cellular function, interacting with cellular structures such as proteins or DNA, inducing the formation of reactive oxygen species (ROS) and free radicals (Figure 2) (Z. Li, Lei, Cheng, & Sun, 2023).

Figure 2. Antibacterial activity of antibiotics encapsulated in liposomes.



Studies in the literature on the administration of liposome-encapsulated antibiotics demonstrate that this therapeutic strategy is more effective than the administration of free antibiotics against infections caused by enterobacteria. Furthermore, given the clinical importance of bacterial resistance, research has been intensified to allow the use of drugs with a limited spectrum of activity against resistant isolates, which can be promoted through liposome encapsulation. Studies that have demonstrated this efficacy are described in Table 1 (Rindhe et al., 2024).

Table 1. Antibacterial activity of liposome-encapsulated antibiotics against enterobacteria.

DRUG	MICROORGANISM	ANTIBACTERIAL ACTIVITY	TYPE OF LIPOSOME	REFERENCES
Gentamicin	Klebsiella oxytoca ATCC 700324	CIMB: 0.25 mg/l	Cationic, Conventional	Alhariri et al., 2017
Cefotaxime + Ellagic Acid	Escherichia coli	Zone of inhibition: 54 ± 2.1 mm	Conventional	Asfour et al., 2017
Polymyxin B	Clinical isolate of A. baumannii	CIMB: 8 ± 2 µg/mL	Chitosan-coated	Fu et al., 2019
Azithromycin	Clinical isolate of E. coli	MIC: 0.62 µg/mL	Conventional	Aljihani et al., 2020
Fusidic Acid	Clinical isolates of Klebsiella pneumoniae Escherichia coli	MIC: 150 µg/ml MIC: 75 µg/mL	Conventional	Nicolosi et al., 2015
Ciprofloxacin, Amikacin, Cloxacillin and Vancomycin	Clinical isolates of MDR K. pneumoniae and E. coli	Zone of inhibition: from 27 to 40 mm	Stealth	Jainamboo et al., 2017
Chloramphenicol	Isolates of Acinetobacter junii and E. coli	The decrease from 127 and 149 UAF	Conventional	Dias-Souza et al., 2017
Azithromycin	Escherichia coli ATCC 8739	MIC50:7.66 µg/mL MIC50: 7.38 µg/ml	Conventional Stealth	Vanić et al., 2019

Nicolosi et al. encapsulated fusidic acid, a bacteriostatic agent, in conventional liposomes and tested its efficacy against clinical isolates of Escherichia coli and Klebsiella pneumoniae. The results demonstrated a MIC of 150 µg/mL for the K. pneumoniae isolate and 75 µg/mL for E. coli, while the MIC value of the free drug was higher than 833 µg/mL for both strains. Therefore, fusidic acid encapsulation showed a higher efficiency due to the ability to transport the drug across the bacterial cell membrane through a fusion mechanism, facilitating the diffusion of the encapsulated drug into the bacteria. Studies indicate that there is a possibility of reducing bacterial resistance through strategies based on the combination of antimicrobial agents co-encapsulated in liposomes. To this end, Asfour co-encapsulated cefotaxime (CXM) and ellagic acid (AE) in conventional liposomes. L-

CXM-AE) and evaluated the antibacterial activity against Escherichia coli isolates and their resistance profile (Xu, Yang, Zhang, Li, & Guo, 2024).

L-CXM-AE had an inhibition zone of 54 ± 2.1 mm, while the formulation without AE had a zone of 51 ± 3.1 mm, thus demonstrating that AE enhances the antibacterial activity of CXM. Moya et al. cefepime encapsulated in cationic liposomes and evaluated the antibacterial activity against Escherichia coli. The LC had a MIC of 2 µg/ml, thus indicating a superiority in the inhibitory capacity of this system compared to the non-encapsulated drug (MIC = 5 µg/ml). Vanić et al. azithromycin encapsulated in conventional liposomes (LC) and propylene glycol-containing liposomes (LPG) and evaluated the antibacterial and antibiofilm activity against Escherichia coli ATCC 8739. LPG showed higher antimicrobial activity with MIC50 of 7.38 µg/mL, whereas the unencapsulated drug obtained MIC50 of 7.50 µg/mL, whereas in LC, the MIC was 7.66 µg/mL (Liñán-Atero et al., 2024).

Aljihani et al. encapsulated the same drug in conventional liposomes (LNA) and evaluated the antibacterial activity against a clinical isolate of Escherichia coli. LNA had a MIC of 0.62 µg/mL, which was more efficient than unencapsulated drugs (MIC = 8-16 µg/mL). Therefore, these studies demonstrate the antibacterial efficacy of azithromycin encapsulation in liposomes. Regarding the antibiofilm potential of liposome-encapsulated drugs, Alhariri, and colleagues encapsulated gentamicin in liposomes made of negatively charged phospholipids and evaluated the antibiofilm activity against Klebsiella oxytoca ATCC 700324. These liposomes had a CIMB of 0.25 mg/L, proving to be more efficient in inhibiting biofilm formation compared to the unencapsulated drug (CIMB = 0.5 mg/L) (She et al., 2023).

This observed inhibition could be the result of a more prolonged release of the drug and a greater penetration capacity into the biofilm, thus allowing greater contact of the drug with bacterial cells. With the same purpose, Fu et al. encapsulated polymyxin B in chitosan-coated liposomes (LPQ) and evaluated the antibiofilm activity, but this time against Acinetobacter baumannii. LPQ showed a CIMB of 8 ± 2 µg/ml and the CIMB of the free drug = 32 ± 2 µg/ml. Therefore, it is suggested that chitosan-coated liposomes interact with the polymeric matrix present in the biofilm, which helps the internalization of the drug and increases the antibiofilm activity (Pant, Kiran, Pande, Mishra, & Dandapat, 2024).

CONCLUSION:

The studies presented in this review demonstrate that the use of liposomes for the delivery of antibiotics for the treatment of bacterial infections is effective. This therapeutic strategy makes the treatment of infections caused by enterobacteria more effective, with a low risk of bacterial resistance and fewer side effects for the patient. Liposomes are now increasingly used in the clinical setting and are highly appreciated due to their biocompatible properties. Some formulations are being analyzed on the market and in clinical trials to improve the treatment of infections caused by enterobacteria.
Given the proven improvement that liposomes promote in antibiotic therapy against enterobacteria, drugs that have a low therapeutic index, high toxicity, and low water solubility are excellent candidates for administration in liposomes. Therefore, if the active ingredients present the characteristics mentioned above, the present work favours the encapsulation of these drugs in liposomes intending to increase the therapeutic arsenal against enterobacteria.

REFERENCES:

1. 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), 396.
2. Allemailem, K. S. (2024). Recent Advances in Understanding the Molecular Mechanisms of Multidrug Resistance and Novel Approaches of CRISPR/Cas9-Based Genome-Editing to Combat This Health Emergency. International Journal of Nanomedicine, 1125-1143.

3. Bolsan, A. C., Sampaio, G. V., Rodrigues, H. C., De Souza, S. S., Edwiges, T., De Prá, M. C., & Gabiatti, N. C. (2024). Phage Formulations and Delivery Strategies: Unleashing the Potential Against Antibiotic-Resistant Bacteria. Microbiological Research, 127662.
4. Bose, S., Sarkar, N., & Jo, Y. (2024). Natural medicine delivery from 3D printed bone substitutes. Journal of Controlled Release, 365, 848-875.
5. Brandelli, A., Lopes, N. A., & Pinilla, C. M. B. (2023). Nanostructured antimicrobials for quality and safety improvement in dairy products. Foods, 12(13), 2549.
6. Caselli, L., Rodrigues, G. R., Franco, O. L., & Malmsten, M. (2023). Pulmonary delivery systems for antimicrobial peptides. Critical Reviews in Biotechnology, 1-18.
7. Drago, L., Minasi, V., Lembo, A., Uslenghi, A., Benedetti, S., Covi, M., . . . Deflorio, L. (2024). Antibiotic Resistance Profiles in Eye Infections: A Local Concern with a Retrospective Focus on a Large Hospital in Northern Italy. Microorganisms, 12(5), 984.
8. Fowoyo, P. T. (2024). Phage Therapy: Clinical Applications, Efficacy, and Implementation Hurdles. The Open Microbiology Journal, 18(1).
9. Gkartziou, F., & Antimisiaris, S. G. (2024). Liposomes for infectious diseases Liposomes in Drug Delivery (pp. 363-404): Elsevier.
10. Hajibonabi, A., Yekani, M., Sharifi, S., Nahad, J. S., Dizaj, S. M., & Memar, M. Y. (2023). Antimicrobial activity of nanoformulations of carvacrol and thymol: New trend and applications. OpenNano, 100170.
11. Johnson, G., Young, M., Gordon, J., & Preuss, C. (2023). Recent Developments in the Treatment of Bacterial Pneumonia. Infectious Diseases Drug Delivery Systems, 275-305.
12. Khambhati, K., Bhattacharjee, G., Gohil, N., Dhanoa, G. K., Sagona, A. P., Mani, I., . . . Jang, S. H. (2023). Phage engineering and phage-assisted CRISPR-Cas delivery to combat multidrug-resistant pathogens. Bioengineering & Translational Medicine, 8(2), e10381.
13. Khater, S. I., Almanaa, T. N., Fattah, D. M. A., Khamis, T., Seif, M. M., Dahran, N., . . . Albedair, R. A. (2023). Liposome-encapsulated Berberine alleviates liver injury in type 2 diabetes via promoting AMPK/mTOR-mediated autophagy and reducing ER stress: morphometric and Immunohistochemical scoring. Antioxidants, 12(6), 1220.
14. Li, Y., Li, X.-m., Duan, H.-y., Yang, K.-d., & Ye, J.-f. (2024). Advances and optimization strategies in bacteriophage therapy for treating inflammatory bowel disease. Frontiers in Immunology, 15, 1398652.
15. Li, Z., Lei, Z., Cheng, D.-B., & Sun, T. (2023). MicroRNA therapeutics and nucleic acid nano-delivery systems in bacterial infection: a review. Journal of Materials Chemistry B.
16. Liñán-Atero, R., Aghababaei, F., García, S. R., Hasiri, Z., Ziogkas, D., Moreno, A., & Hadidi, M. (2024). Clove Essential Oil: Chemical Profile, Biological Activities, Encapsulation Strategies, and Food Applications. Antioxidants, 13(4), 488.
17. Liu, K., Wang, C., Zhou, X., Guo, X., Yang, Y., Liu, W., . . . Song, H. (2024). Bacteriophage therapy for drug-resistant Staphylococcus aureus infections. Frontiers in Cellular and Infection Microbiology, 14, 1336821.
18. Manicum, A.-L. E., Makgopa, K., Shabangu, T., Kumar, G. V., Agwamba, E. C., Shai, L. J., & Ghotekar, S. (2023). Nanotechnology-based therapeutics to combat biofilms and antibacterial resistance in chronic wound infections Bioengineered Nanomaterials for Wound Healing and Infection Control (pp. 175-206): Elsevier.
19. Mehmood Khan, F., Manohar, P., Singh Gondil, V., Mehra, N., Kayode Oyejobi, G., Odiwuor, N., . . . Huang, G. (2023). The applications of animal models in phage therapy: an update. Human Vaccines & Immunotherapeutics, 19(1), 2175519.
20. Mohanty, D., Suar, M., & Panda, S. K. (2023). Nanotechnological interventions in bacteriocin formulations–advances, and scope for challenging food spoilage bacteria and drug-resistant foodborne pathogens. Critical Reviews in Food Science and Nutrition, 1-18.
21. Pant, M., Kiran, K., Pande, V., Mishra, B., & Dandapat, A. (2024). Use of nano biotechnological methods for the analysis and stability of food antimicrobials and antioxidants Nanobiotechnology for Food Processing and Packaging (pp. 449-480): Elsevier.

22. Panthi, V. K., Fairfull-Smith, K. E., & Islam, N. (2024). Liposomal drug delivery strategies to eradicate bacterial biofilms: Challenges, recent advances, and future perspectives. International Journal of Pharmaceutics, 124046.
23. Paudel, S., Apostolakos, I., Vougat Ngom, R., Tilli, G., de Carvalho Ferreira, H. C., & Piccirillo, A. (2024). A systematic review and meta-analysis on the efficacy of vaccination against colibacillosis in broiler production. PLoS ONE, 19(3), e0301029.
24. Pinilla, C. M. B., Lopes, N. A., & Brandelli, A. (2023). Liposome-mediated encapsulation of antimicrobials and probiotics Liposomal Encapsulation in Food Science and Technology (pp. 65-86): Elsevier.
25. Plotniece, A., Sobolev, A., Supuran, C. T., Carta, F., Björkling, F., Franzyk, H., . . . De Vooght, L. (2023). Selected strategies to fight pathogenic bacteria. Journal of enzyme inhibition and medicinal chemistry, 38(1), 2155816.
26. Pushpalatha, C., Venkataramana, S., Ramesh, P., Kavya, B., Nagaraja, S., & Kumar, K. V. (2024). Nanoparticles to Abate Antibiotic Resistance During the Management of Dental Diseases Nanotechnology Based Strategies for Combating Antimicrobial Resistance (pp. 453-487): Springer.
27. Pylypenko, D., Grigoryeva, G., & Krasnopolsky, Y. M. (2023). PROSPECTS FOR THE CREATION OF LIPOSOMAL ANTIMICROBIALS BASED ON PHAGES. Biotechnologia Acta, 16(5), 22-33.
28. Rajangam, S. L., & Narasimhan, M. K. (2024). Current treatment strategies for targeting virulence factors and biofilm formation in Acinetobacter baumannii. Future Microbiology, 1-21.
29. Rindhe, S., Khan, A., Priyadarshi, R., Chatli, M., Wagh, R., Kumbhar, V., . . . Rhim, J. W. (2024). Application of bacteriophages in biopolymer-based functional food packaging films. Comprehensive Reviews in Food Science and Food Safety, 23(3), e13333.
30. Roy, S., Hasan, I., & Guo, B. (2023). Recent advances in nanoparticle-mediated antibacterial applications. Coordination Chemistry Reviews, 482, 215075.
31. Saxena, D., Maitra, R., Bormon, R., Czekanska, M., Meiers, J., Titz, A., . . . Chopra, S. (2023). Tackling the outer membrane: facilitating compound entry into Gram-negative bacterial pathogens. npj Antimicrobials and Resistance, 1(1), 17.
32. Scoffone, V. C., Barbieri, G., Irudal, S., Trespidi, G., & Buroni, S. (2024). New Antimicrobial Strategies to Treat Multi-Drug Resistant Infections Caused by Gram-Negatives in Cystic Fibrosis. Antibiotics, 13(1), 71.
33. She, P., Li, Y., Li, Z., Liu, S., Yang, Y., Li, L., . . . Wu, Y. (2023). Repurposing 9-aminoacridine as an adjuvant enhances the antimicrobial effects of rifampin against multidrug-resistant Klebsiella pneumoniae. Microbiology Spectrum, 11(3), e04474-04422.
34. Torres Di Bello, D., Narváez, D. M., Groot de Restrepo, H., & Vives, M. J. (2023). Cytotoxic Evaluation in HaCaT Cells of the Pa. 7 Bacteriophage from Cutibacterium (Propionibacterium) acnes, Free and Encapsulated Within Liposomes. PHAGE, 4(1), 26-34.
35. Tripathi, A. K., Singh, J., Trivedi, R., & Ranade, P. (2023). Shaping the Future of Antimicrobial Therapy: Harnessing the Power of Antimicrobial Peptides in Biomedical Applications. Journal of Functional Biomaterials, 14(11), 539.
36. Wang, B., Du, L., Dong, B., Kou, E., Wang, L., & Zhu, Y. (2024). Current Knowledge and Perspectives of Phage Therapy for Combating Refractory Wound Infections. International Journal of Molecular Sciences, 25(10), 5465.
37. Wang, D.-Y. (2023). A self-targeting liposomal drug carrier with water as a pH-responsive functionality.
38. Xu, W., Yang, T., Zhang, J., Li, H., & Guo, M. (2024). Rhodiola rosea: a review in the context of PPPM approach. EPMA Journal, 1-27.
39. Yan, C., & Kim, S.-R. (2024). Microencapsulation for pharmaceutical applications: a review. ACS Applied Bio Materials, 7(2), 692-710.

40. Yang, J., Zhu, X., Xu, X., & Sun, Q. (2023). Recent knowledge in phages, phage-encoded endolysin, and phage encapsulation against foodborne pathogens. Critical Reviews in Food Science and Nutrition, 1-21.
41. Yao, J., Zou, P., Cui, Y., Quan, L., Gao, C., Li, Z., . . . Yang, M. (2023). Recent advances in strategies to combat bacterial drug resistance: antimicrobial materials and drug delivery systems. Pharmaceutics, 15(4), 1188.
42. Zhang, Y., Sharma, S., Tom, L., Liao, Y.-T., & Wu, V. C. (2023). Gut phagosome—An insight into the role and impact of gut microbiome and their correlation with mammal health and diseases. Microorganisms, 11(10), 2454.