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REVIEW ARTICLE

The role of efflux pumps in microbial resistance: an approach to the topic

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ABSTRACT

Introduction: bacterial resistance to antibiotics constitutes a global threat to public health, driven by adaptive mechanisms such as efflux pumps.
Objective: to describe the role of efflux pumps in microbial resistance and their involvement in multidrug-resistant strains.
Methods: a systematic review of the scientific literature was conducted across different databases, using a keyword algorithm with Boolean operators to identify relevant sources. The selected studies, after applying rigorous inclusion and exclusion criteria, were critically evaluated in terms of timeliness, methodological quality, and thematic relevance, and coherently integrated into the final synthesis of the review.
Development: efflux pumps are membrane proteins that expel antibiotics out of the cell, reducing their internal concentration. They are classified into families such as ATP-binding cassette transporters, major facilitators, resistance-nodulation-division, small multidrug resistance, and multidrug and toxic compound extrusion. These structures are present in Gram-positive and Gram-negative bacteria, including pathogens such as Staphylococcus aureus, Escherichia coli, and Acinetobacter baumannii. They are also associated with biofilm formation and antimicrobial tolerance. Research on efflux pump inhibitors suggests promising therapeutic alternatives, although no drugs have yet been approved for clinical use.
Conclusions: efflux pumps are key mechanisms in bacterial resistance and in the emergence of multidrug-resistant strains. Their study opens possibilities for designing inhibitors that enhance the effectiveness of existing antibiotics.

Keywords: Drug Resistance, Microbial; Bacterial Proteins; Drug Resistance, Multiple.

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INTRODUCTION

The discovery of antibiotics revolutionized medicine by providing effective therapies against bacterial infections. However, their indiscriminate use and the evolutionary pressure exerted on microorganisms have favored the emergence of resistant strains, now recognized as a global public health threat. Antibiotic resistance is a complex phenomenon underpinned by diverse mechanisms, among which efflux pumps stand out—membrane proteins that enable bacteria to expel antimicrobials and reduce their efficacy. These systems, present in multiple pathogens, reflect the rapid adaptive capacity of bacteria and underscore the need for specific scientific responses to ensure treatment effectiveness and patient safety.[1]

To address this growing threat, it is imperative to develop new therapeutic strategies and tackle the problem through coordinated global action. Essential measures include research into alternative therapies, development of rapid diagnostic methods to initiate targeted antibiotic treatment early, implementation of global vaccination programs, educational campaigns, improved infection control practices, and support for transitioning to animal husbandry and production systems with reduced antibiotic consumption. [2]

Current scientific literature reflects growing concern over the spread of antibiotic resistance, highlighting the significant contribution of efflux pumps in this scenario. Numerous studies have identified the presence and function of these membrane proteins in various bacterial strains, demonstrating their active role in antibiotic expulsion and consequent reduction in therapeutic efficacy. Efflux-mediated resistance not only affects a broad spectrum of antibiotics but is also associated with the emergence of multidrug-resistant strains, further complicating the clinical management of infections. [3]

A critical analysis of prior work underscores the complexity of this phenomenon. Each efflux pump comprises three fundamental components: the outer membrane channel, the periplasmic lipoprotein, and the inner membrane transporter. Antimicrobial resistance in *Acinetobacter baumannii* is linked to four distinct efflux pump classes: the major facilitator superfamily (MFS), the resistance-nodulation-division (RND) family, the small multidrug resistance (SMR) family, and the multidrug and toxic compound extrusion (MATE) family. Although multiple pumps play a role, detailed research has primarily focused on the MFS and RND transporter families. [4]

Both AdeABC and RND-type efflux pumps contribute not only to aminoglycoside resistance but also to resistance against a variety of other antibiotics, including tigecycline, β-lactams, chloramphenicol, erythromycin, and tetracycline. Among the five superfamilies of efflux pumps, resistance-nodulation-division (RND) systems are particularly significant in the context of multidrug-resistant *A. baumannii*. These systems exhibit a broad substrate range, including antibiotics, dyes, biocides, detergents, and antiseptics. [5,6]

Efflux pumps not only expel antibiotics from bacterial cells but may also be encoded by genes whose expression is induced by antibiotic presence. This creates a vicious cycle that perpetuates bacterial resistance, as continuous antibiotic exposure stimulates the production and activity of these pumps. Furthermore, considerable variability in the presence and activity of these pumps has been observed across different bacterial strains and species, emphasizing the need for specific, context-adapted approaches in clinical settings. [7] In this context, the present study aims to describe the role of efflux pumps in microbial resistance and their implications in multidrug-resistant strains. The scope of this research focuses on understanding the function and relevance of these pumps in bacterial antibiotic resistance, considering the genetic and phenotypic variability of bacteria.

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METHODS

A systematic literature review was conducted in accordance with PRISMA 2020 guidelines to analyze the role of efflux pumps in bacterial antibiotic resistance. The search period was limited to 2018–2024, focusing on recent studies addressing molecular and clinical mechanisms.

Information sources included SciELO, Elsevier, Google Scholar, and PubMed, as well as gray literature and secondary references. Articles in English and Spanish were considered to ensure international coverage. The search strategy employed MeSH and DeCS terms: "efflux pumps," "antibiotic resistance," "multidrug-resistant bacteria," "resistance mechanisms," combined with Boolean operators.

Inclusion criteria comprised original articles, systematic reviews, and experimental studies specifically addressing the role of efflux pumps in bacterial resistance. Studies without full-text access, duplicates, and those lacking information on resistance mechanisms were excluded. A rigorous selection process ensured methodological transparency. A reduced set of key articles was ultimately included based on relevance and methodological quality.

Data extraction and analysis focused on variables such as efflux pump type, implicated bacteria, affected antibiotics, and associated genetic mechanisms. A qualitative synthesis of findings was performed and organized chronologically to illustrate the evolution of knowledge in the field.

RESULTS

Since the discovery of the first drug-resistant efflux pump in the 1990s, advances in molecular microbiology have led to the characterization of numerous efflux pumps in Gram-positive bacteria—including methicillin-resistant *Staphylococcus aureus* and *Streptococcus pneumoniae*—and in Gram-negative bacteria such as *Acinetobacter baumannii*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Stenotrophomonas maltophilia*. As these pumps transport substrates against a concentration gradient, they are energy-dependent. Based on their energy acquisition mechanism, efflux pumps are broadly classified into two categories: primary pumps, which obtain energy through active ATP hydrolysis, and secondary pumps, which harness energy from proton or sodium ion gradients. [4]

The WHO's identification of priority antibiotic-resistant bacteria highlights the urgency for action. These strategies, coupled with awareness of antibiotic resistance as a public health issue, are key to mitigating the impact of resistant infections. International cooperation and concrete measures are essential to preserve the efficacy of medical treatments and safeguard global health. The need for a coordinated, global approach to therapeutic research and development becomes evident given the scale of this emerging health challenge. [8]

Given the importance of efflux pumps in mediating antibiotic resistance, it is reasonable to expect that circumventing these resistance determinants could enhance the activity of substrate antibiotics. Efflux abolition could be achieved through several approaches:[4]

- Regulating efflux pump gene expression by interfering with genetic regulation
- Redesigning antibiotics so they are no longer recognized as substrates
- Inhibiting the assembly of functional efflux pumps
- Blocking the pump to prevent substrate binding at the active site
- Collapsing the energy mechanism that powers these pumps

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The ATP-binding cassette (ABC) superfamily comprises primary transporters that directly use energy from ATP binding and hydrolysis to export solutes from bacterial cells. Structurally, these transporters feature transmembrane domains (TMDs) with substrate-binding regions and nucleotide-binding domains (NBDs), where ATP hydrolysis drives the transport cycle. They are classified as homodimeric or heterodimeric, with the latter being especially important in antibiotic resistance in Gram-positive bacteria. Heterodimeric ABC transporters possess a single nucleotide-binding site that does not participate in ATP hydrolysis, providing a valuable feature for differentiation from homodimeric types. (9)

The major facilitator superfamily (MFS) represents the largest and most diverse family of transporters. Its members are substantial in size, functioning as monomeric units with transmembrane helix domains. They are classified as uniporters, symporters, or antiporters based on their coupling with ionic gradients during transport. Their presence across all domains of life underscores their biological relevance. The multidrug and toxic compound extrusion (MATE) family is organized into subfamilies such as NorM, DinF, and eukaryotic variants. MATE transporters feature two six-transmembrane-helix bundles with a topology distinct from that of the MFS superfamily. Bacterial MATE transporters utilize proton-motive force or Na^+ gradients to export polycyclic aromatic and cationic drugs. (4,9)

The resistance-nodulation-cell division (RND) superfamily is prominent in Gram-negative bacteria and highly conserved across species. RND proteins are larger and form homotrimers. Clinically significant bacteria such as *Acinetobacter baumannii* and *Pseudomonas aeruginosa* employ these efflux pumps to resist a wide array of antibiotics. The small multidrug resistance (SMR) family includes compact efflux pumps that confer resistance to quaternary ammonium compounds and lipophilic cationic molecules. Examples include *Acinetobacter baumannii* using the AbeS pump to resist chloramphenicol, ciprofloxacin, and erythromycin; *Mycobacterium tuberculosis* employing the Mmr pump against ofloxacin and rifampicin; and *Neisseria gonorrhoeae* exhibiting resistance to aminoglycosides, penicillin, azithromycin, and ceftriaxone via the EmrE transporter. (9,10)

Antimicrobial tolerance mediated by biofilms represents a significant challenge, particularly in healthcare-associated infections. Biofilms—characterized by an exopolysaccharide matrix and persistent bacterial subpopulations—employ multiple resistance mechanisms, including quorum sensing, porins, efflux pumps, gene expression modulation, membrane vesicles, extracellular DNA, and enzymes. This review seeks to identify the specific mechanisms and effects of *Pseudomonas aeruginosa* biofilms in antibiotic resistance. Biofilm-associated bacterial tolerance to antibiotics is crucial: minimal antibiotic exposure induces specific gene expression within these microbial structures, reducing antibiotic penetration and efficacy. *P. aeruginosa* grown as a biofilm exhibits up to 1,000-fold greater antibiotic tolerance compared to its planktonic form—a phenomenon primarily linked to mechanisms such as quorum sensing, porins, efflux pumps, gene regulation, membrane vesicles, extracellular DNA, and enzymatic activity. (1)

Quorum sensing—a cell-to-cell communication mechanism—enables biofilm bacteria to adapt to microenvironmental changes. Mediated by signaling molecules called autoinducers, this process coordinates gene expression in response to bacterial population density. Acyl-homoserine lactone– and quinolone-based systems, such as the *Pseudomonas* quinolone signal (PQS), play crucial roles. Efflux pumps, integral to biofilms, significantly contribute to survival and antibiotic tolerance by expelling antimicrobials from bacterial cells, thereby supporting both intrinsic and acquired tolerance. Overproduction of these pumps—combined with target site modification and antibiotic inactivation—generates a tolerance phenotype inducible by various biofilm stressors, including oxidative or nitrosative stress. (11)

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Several studies demonstrate that efflux pumps significantly contribute to biofilm formation—a complex phenomenon in which these pumps play a key role. Thus, inhibiting efflux pumps could represent a promising strategy to enhance antibiotic activity, especially as the discovery of new antibiotics becomes increasingly difficult. Efflux pump inhibitors (EPIs) are molecules designed to block efflux pump activity and are considered potential therapeutic agents to restore the efficacy of antibiotics rendered ineffective by resistance. However, specific substrates for many pumps remain undetermined, and research continues into how altered pump activity affects biofilm formation. (12)

Reviving the activity of antimicrobials no longer effective against bacterial pathogens like *Pseudomonas aeruginosa* has been proposed as a strategy to combat antimicrobial resistance. Novel EPIs have demonstrated significant potentiation when combined with levofloxacin against wild-type *P. aeruginosa* ATCC 27853. Structure–activity relationships of aryl-substituted heterocyclic carboxamides containing a pentane diamino side chain have been described. Among fused heterocyclic carboxamides, the indole aryl carboxamide compound 6j (TXA01182) showed 8-fold levofloxacin potentiation at 6.25 µg/mL. TXA01182 also exhibited synergistic activity with other antimicrobial classes against *P. aeruginosa* and demonstrated synergy with levofloxacin against multiple clinical multidrug-resistant *P. aeruginosa* strains. This approach represents a promising strategy to overcome antimicrobial resistance, offering new tools against resistant pathogens and highlighting the importance of EPIs. (13)

High mortality and morbidity associated with tuberculosis (TB)—particularly in developing countries—are key factors that characterize TB as a major global public health problem. In the absence of a more effective vaccine, chemotherapy remains a primary TB control tool. Multidrug-resistant TB (MDR-TB) is caused by *M. tuberculosis* strains resistant to at least rifampicin and isoniazid—two cornerstone drugs in TB treatment. Although efflux mechanisms have been studied in various mycobacteria, *M. smegmatis* has served as the model system for expressing heterologous efflux pump genes and studying efflux mechanisms. The first efflux pump described in mycobacteria was LfrA—a major facilitator superfamily (MFS) protein in *M. smegmatis*—which, when expressed from multicopy plasmids, confers low-level resistance to fluoroquinolones, ethidium bromide, acridine, and certain quaternary ammonium compounds. Other initially characterized mycobacterial efflux pumps include TetV (conferring tetracycline resistance) and Tap (Rv1258c), which, when overexpressed in *M. smegmatis*, confers low-level resistance to aminoglycosides and tetracycline. (14)

The SMR (small multidrug resistance) family of efflux pumps confers resistance in archaea and bacteria against antibiotics resembling quaternary ammonium molecules and other lipophilic cationic compounds. These pumps are extremely small, featuring only four transmembrane helices (TMHs), and typically function as homodimers or heterodimers. A classic example is EmrE in *E. coli*. Structural analyses of this protein using X-ray crystallography, cryo-electron microscopy (cryo-EM), and nuclear magnetic resonance (NMR) have revealed an antiparallel protomer arrangement. (9)

The ability of EPIs to reverse antibiotic resistance is a crucial point warranting close attention. At a time when bacterial resistance has become a global threat, leveraging the established pharmacological properties of existing antibiotics offers a potentially efficient and cost-effective solution. This strategy not only saves time and resources associated with developing new antibiotics but could also provide rapid, effective responses to otherwise untreatable infections.(4)

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Combining antibiotics with EPIs addresses not only already-resistant bacteria but also serves as a proactive defense against future resistance. This strategic approach implies not only the search for new antibiotics but also the optimization and maximization of the therapeutic potential of existing ones. However, significant challenges must be addressed to ensure the safety and efficacy of this emerging strategy. (10)

In developing broad-spectrum EPIs, several concerns must be considered. First, efflux pumps provide protection only in actively growing bacterial cells, potentially limiting their efficacy against slow-growing or non-replicating pathogens. This raises questions about the generalized applicability of this strategy and highlights the need for personalized approaches based on infection characteristics. Additionally, a critical concern involves ATP-dependent efflux pumps present in human cells—particularly those overexpressed in multidrug-resistant cancer cells, which are designed to prevent toxicity from anticancer agents. (14)

Despite substantial efforts to discover new antibiotic adjuvants acting as EPIs—and despite promising results—it is regrettable that no EPIs are currently approved for human or veterinary use, nor are any in clinical trials. This gap underscores the need for greater investment and commitment to research and development in this area.

Ultimately, the discussion on bacterial resistance and the role of EPIs leads to a broader reflection on infection control strategies. Since the discovery of penicillin, tactics have evolved slowly, with most efforts still focused on discovering new antibiotic classes. However, persistent bacterial resistance demands a paradigm shift. It is imperative not only to seek new options but also to extend the lifespan of existing antibiotics and simultaneously address bacterial resistance mechanisms in a more comprehensive manner.(15)

CONCLUSIONS

Efflux pumps represent a key strategy in combating antibiotic resistance, as their role across families such as ABC, MFS, MATE, RND, and SMR illustrates how continuous drug exposure can promote bacterial resistance. In this context, efflux pump inhibitors (EPIs) emerge as a promising alternative—though still without clinical approval—highlighting the need for research and development to combine them with existing antibiotics and enhance efficacy against multidrug-resistant strains. The exploration of synthetic compounds (quinolines, indoles, pyridines, phenols, sulfur-containing heterocycles), natural products, and machine learning tools reflects the diversity and dynamism of this field, while challenges related to specificity, safety, and pharmacokinetic adaptation demand rigorous evaluation. Ultimately, advancing toward more effective combination therapies requires innovation and interdisciplinary collaboration to translate these findings into safe, sustainable clinical solutions against antibiotic resistance.

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