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The Action of Efflux Pump Genes in Conferring Drug Resistance to *Klebsiella* Species and Their Inhibition

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Abstract

Nosocomial infections caused by *Klebsiella* species are characterized by high rates of morbidity and mortality. The emergence of the multidrug-resistant (MDR) and extensive drug-resistant (XDR) Gram-negative bacteria reduces the antibiotic efficacy in the treatment of infections caused by the microorganisms. Management of these infections is often difficult, due to the high frequency of strains resistant to multiple antimicrobial agents. Multidrug efflux pumps play a major role as a mechanism of antimicrobial resistance in Gram-negative pathogens. Efflux systems are significant in conferring intrinsic and acquired resistance to the bacteria. The emergence of increasing drug resistance among *Klebsiella pneumoniae* nosocomial isolates has limited the therapeutic options for treatment of these infections and hence there is a constant quest for an alternative. In this review, we discuss various resistance mechanisms, focusing on efflux pumps and related genes in conferring resistance to *Klebsiella*. The role of various efflux pump inhibitors (EPIs) in restoring the antibacterial activity has also been discussed. In specific, antisense oligonucleotides as alternative therapeutics in combatting efflux-mediated resistance in *Klebsiella* species have focused upon.

Keywords
- efflux pump systems
- *K. pneumoniae*
- efflux pump inhibitors
- antisense oligonucleotides

Introduction

Enterobacteriaceae, in particular, *Klebsiella*, is one of the outstanding etiological agents of significant importance in causing nosocomial and community-associated infections in humans.[1] Bacteria belonging to the genus *Klebsiella* cause a wide range of infections such as community-acquired infections including pneumonia, wound infections, urinary tract infections, septicemia, and gastrointestinal diseases.[2] Recognized species of the genus *Klebsiella* include *K. pneumoniae*, *K. oxytoca*, *K. terrigena*, *and K. planticola*.[3] *K. pneumoniae* is the principal species of the genus, gaining importance as it is developing multidrug resistance in hospital settings similar to *Acinetobacter* and *Pseudomonas*.[4] The source of isolation of *K. oxytoca* from clinical specimens includes blood and respiratory secretions. It causes infections in the immunocompromised individuals admitted in intensive critical care units. In the history of therapeutics, one of the major milestones was the discovery of antibiotics, which no doubt was effective in controlling infectious agents and considered a wonder drug. Yet, extensive use of antibiotics in the medical, agricultural, and veterinary sectors has led to the emergence of drug-resistant strains. Listed as one of the ESKAPE (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa*, and *Enterobacter* species) pathogens, *Klebsiella* has gained resistance to almost all the drugs.[5] The possible reasons for frequent infections caused by *Klebsiella* in comparison to other Gram-negatives could be (a) the ability to naturally resist the antibiotics, (b) the ability to outcompete

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other bacteria, (c) the potential to withstand starvation, (d) the capability to exchange genetic material readily with other members of the human microbiome, and (e) mobile genetic elements that encode broad-spectrum of virulence and antibiotic genes.[6,7] Utmost strains of *K. pneumoniae* produce the capsular polysaccharide, conferring resistance to antimicrobial peptides and phagocytosis of the host immune response. Mobile genetic elements play a crucial role in the dissemination of several factors that play an essential role in the spread of drug-resistant *Klebsiella*.[8] *K. pneumoniae* surpasses the host immune responses via several virulence genes harbored such as siderophores, fimbriae, adhesins, lipopolysaccharide (LPS), and outer membrane proteins for the survival and immune evasion during infection.[5] The potential of the resistant bacteria and its genes to survive the hospital sewage water treatments contribute to the spread and act as a reservoir of antimicrobial resistance causing constant risks to humans and animals.[9] The bacteria can be directly transmitted via direct contact, contaminated environments, and medical equipment are in patients affected with *Klebsiella* infections.

Drug Resistance

Under constant antibiotic pressure, the bacteria acquire antimicrobial resistance genes via mutations, plasmids, and transferable genetic elements resulting in the multidrug-resistant and extremely drug-resistant strains (XDR) possessing a "super resistome."[5] This evolution over the years has enabled the bacteria to gain resistance to all possible antibiotics with no treatment options left behind.[10] The likelihood of dissemination of these drug-resistant pathogens is recognized as a global threat. In the era of drug resistance, *K. pneumoniae* is one of the most alarming nosocomial pathogens associated with antibiotic resistance, labeled as a significantly important MDR strain and listed under the ESKAPE groups of pathogens.[11] *Klebsiella* species harbor plasmids responsible for resistance to β-lactams, specifically extended-spectrum cephalosporins and also carbapenems.[12] All these make the treatment options limited and end up in the usage of last line of drugs like fluoroquinolones. Unfortunately, *K. pneumoniae* resistance to fluoroquinolones has drastically increased and has been reported in the recent past. Resistance toward fluoroquinolones occurs due to specific mutations in the DNA gyrase, topoisomerase IV, and overexpression of the multidrug efflux system.[13] The European Antimicrobial Resistance Surveillance Network data for the years 2005 to 2015 demonstrate the *K. pneumoniae* nonsusceptibility toward four major classes of drugs: (a) carbapenems, (b) third-generation cephalosporins, and (c) fluoroquinolones and aminoglycosides (http://atlas.ecdc.europa.eu/public/index.aspx?Instance). Multifactorial dissemination of the resistance genes increases the occurrence of the MDR and XDR strains of *K. pneumoniae*. In this regard knowledge on various mechanisms of resistance conferred by the bacteria to various drugs has been described in this review. However, the main focus is on efflux-mediated resistance and alternatives to combat this particular mode of resistance in *Klebsiella*.

As stated earlier, development of resistance in bacteria is an evolutionary process. Resistance to antibiotics could be developed either by spontaneous mutations or through the acquisition of resistance genes. Drug resistance can evolve through several mechanisms. Two broadly categorized mechanisms are the intrinsic (innate) and the acquired resistance. The acquired resistance may be due to the (a) acquisition of exogenous genes by conjugation (transposons) or transformation (plasmids) and transduction (integrons and bacteriophages), (b) mutation of cellular genes, and (c) combination of these mechanisms.[14]

Plasmid-Mediated Resistance

In general, the plasmids are double-stranded deoxyribonucleic acid (DNA) encoding almost 10% of the host cell chromosome.[15] In conferring resistance to toxic heavy metals, antimicrobial agents, and virulence determinants, transfer of genes is very efficient in helping the bacterial cell survive in the environment regardless of the lethal antibiotic dose.[14]

Transposons and Insertion Elements

The multidrug resistance genes located in a DNA sequence can be transferred from one plasmid to the other, or between genomes via transposon (jumping gene) systems.[16] The insertion (IS) elements have terminal repeat sequences and can recognize a protein necessary to insert or remove a transposon from a specific region.[17] The integrons (gene capture) systems are also responsible for conferring resistance and drive the expression of resistance genes by the use of a specific recombination mechanism. The three main components of the integrons encoded in the conserved 5' segment are (i) enzyme integrase (*int*) that serves as a specific recombination system to insert or remove a new gene cassette, (ii) a specific recombination site (*attI* site), and (iii) a promoter to initiate gene transcription. Nearly all the integrons of class I in the 3' conserved segment have a supplementary gene (*sulI*) responsible for conferring resistance to sulphonamide.[18]

Mutations are the genetic changes occurring naturally, yet influence the bacteria to sustain and grow under extreme environmental pressure (antimicrobials).[19] The selection of mutants involves factors such as the size of the bacterial population, immune status of the host, presence of other microbiota.[20] Frequently, random mutations of the genes that encode antibiotic lytic enzymes generate modified catalysts resulting in an increased spectrum of antibiotic resistance enzymes.

Efflux Pumps and Outer Membrane Permeability

Besides enzymatic modifications, overexpression of the MDR efflux systems substantially contribute to drug resistance. Efflux pumps, specifically the multidrug efflux, is of major concern to the drug therapy as they expel a wide range of substrates and clinically significant drugs.[21,22] Efflux pumps are dominant in mediating resistance to diverse classes of

drugs and biofilm structures.[23] The efflux pumps not only extrude the given antibiotics out of the bacterial cell but also act as virulence factors and adaptive responses that bestow antimicrobial resistance during infections. In brief, these efflux systems allow the microorganisms in balancing the internal environment by extruding the toxic substances, such as metabolites, antimicrobial agents, and quorum-sensing-regulated expression of virulence determinant. So far, *acr*AB, *oqx*AB, and *kex*D efflux systems have been associated with the antibiotic resistance of *K. pneumoniae.* In addition to *acr*AB, *kex*D, *kde*A, *kmr*A, *kpn*EF, and *oqx*AB few other efflux genes such as *Eef*AB, *ket*M, and *Cep*A are also reported in *K. pneumoniae*.[24,25]

Several efflux systems have so far been functionally characterized—(a) resistance nodulation division (RND) family (*acr*AB and *kex*D), (b) multidrug and toxic compound extrusion family (MATE) (*kde*A) efflux gene, (c) major facilitator superfamily (MFS) (*kmr*A) gene, and (d) small MDR family (*kpn*EF). Schematic representation of different efflux systems in Gram-negative bacteria is shown in ►**Fig. 1**.

RND-type Efflux Pump

Reports demonstrate overexpression of the RND-type efflux pump in the clinical isolates of *K. pneumoniae*. The RND type of efflux system consists of three components: inner membrane protein, periplasmic protein, and outer membrane protein. An inner component consists of a multisite binding pocket and recognizes several chemicals and the respective substrates.[26] A trimeric complex formed by the protein component gains access, binds, and finally expels out the substrates. Besides its role in intrinsic resistance toward a wide range of drugs, the RND efflux system facilitates the bacteria in colonizing the gastrointestinal tract and release virulence factors.[27] Majority of them describe the overexpression of AcrB and OqxAB increases the opportunity of multidrug resistance in *K. pneumoniae*.[28] AcrAB efflux system in *K. pneumoniae* is encoded by acrRAB operon, wherein the *acr*R encodes the AcrAB repressor, while the *acr*A and *acr*B encode a periplasmic lipoprotein attached to the inner membrane connecting the outer, inner membranes, and an integral membrane protein situated in the cytoplasmic membrane.[29] Resistance to quinolones (ciprofloxacin and nalidixic acid) and other antibiotics (erythromycin, cefoxitin, tigecycline, and chloramphenicol) is associated with the multidrug efflux system AcrRAB. The efflux system is also associated with conferring resistance to antimicrobial peptides in the lungs.[27]

OqxAB Efflux System

Resistance toward chloramphenicol, nalidixic acid, cefoxitin, and ciprofloxacin is related to the OqxAB efflux system. It is a part of the rarA-oqxABR locus, wherein RarA acts as a transcriptional regulator of oqxAB and OqxR acting as a transcriptional repressor of *oqx*AB and *rar*A.[30] KpnEF efflux pump in *K. pneumoniae* confers resistance to several dyes, detergents, and antimicrobial compounds such as benzalkonium chloride, ceftriaxone, chlorhexidine, acriflavine, cefepime,

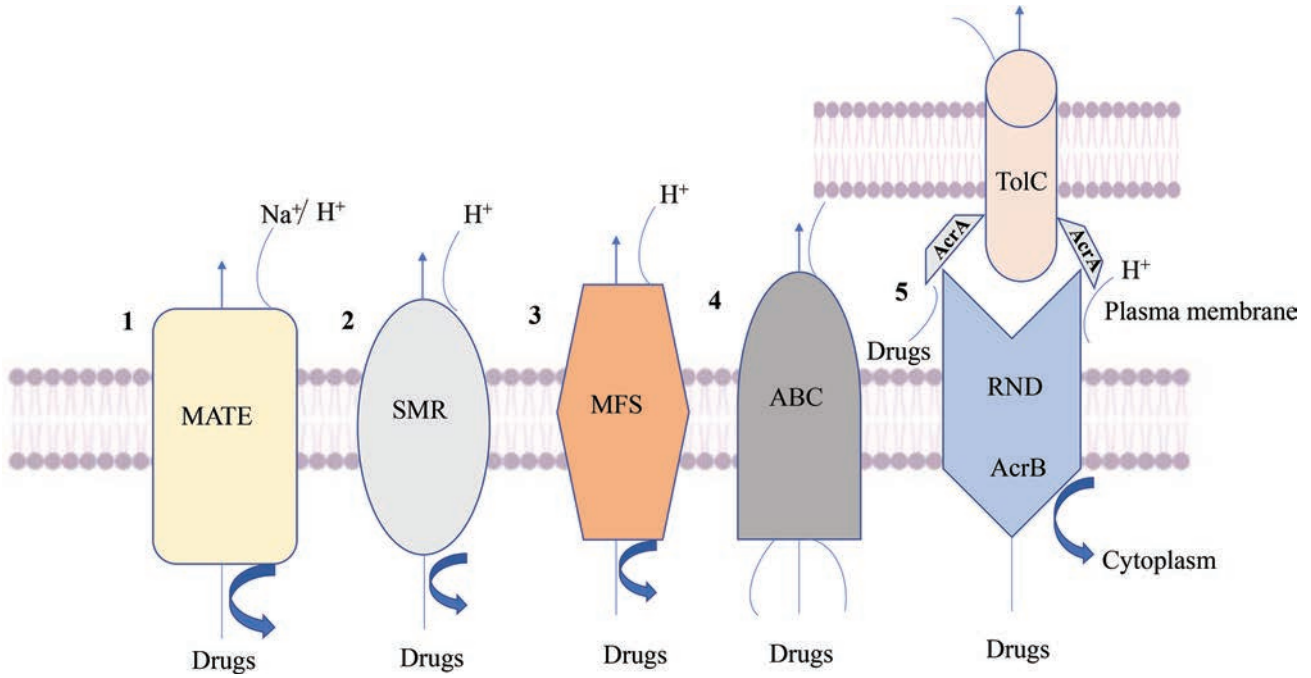

Fig. 1 Schematic representation of different efflux systems in Gram-negative bacteria. (1) MATE: multidrug and toxic compound extrusion superfamily, (2) SMR: small multidrug resistance protein, (3) MFS: major facilitator superfamily, (4) ABC: ATP- binding cassette transporter, and (5) RND: resistance nodulation division superfamily.

colistin, erythromycin, streptomycin, rifampin, triclosan, and tetracycline.[31]

Other Related Efflux Systems

A subset of inner membrane proteins concerned with the MFS function as efflux pumps, decrease the intracellular concentration of a broad spectrum of drugs and chemicals, thus conferring resistance to the bacteria.[32] The efflux pump *Eef*AB, though not associated with the antimicrobial drug resistance, was observed to colonize the murine digestive tract[25]; *ket*M was not significantly found related to contributing toward resistance to antibiotics.

In *K. pneumoniae* loss of outer membrane protein in syncronization with the acquisition of ampC β-lactamase and new generation carbapenemase A confers resistance to carbapenems and aminoglycosides, and reduced sensitivity toward fluoroquinolones, tetracycline, chloramphenicol, erythromycin, trimethoprim, ethidium bromide, and meropenem.[15] Mutations in the quinolone resistance-determining region in *K. pneumoniae* and *K. oxytoca* are associated with the resistance phenotypes.[33]

Efflux Pump Inhibitors

Inhibiting these efflux pumps might seem like an effective strategy at times when the conventional antibiotics remain no longer effective. Establishment of natural substrates and efflux pump inhibitors (EPIs), that enable effective accumulation of drug inside the bacterial cell, enhances the antibacterial activity. Some of the plant-derived EPIs used are reserpine, pipeline, catechin gallates, flavonoids, and geraniol.[34] Frequently used synthetic efflux inhibitors to detect the efflux activity in *Klebsiella pneumoniae* are carbonyl cyanide-chlorophenylhydrazone (CCCP) and phenylalanine-arginine β-naphthylamide (PAßN).[35] General mechanism of EPIs is depicted in ►**Fig. 2**.[36] Many studies have demonstrated the efficacy of the efflux inhibitors in inducing sensitivity to the bacteria, which still restores hope within us.

Fang et al[37] exposed the *K. pneumoniae* carbapenemase-producing tigecycline-resistant isolates to 1-(1-naphthylmethyl)-piperazine (NMP). On exposure to EPI *K. pneumoniae* isolates previously resistant to multiple drugs showed an obvious decrease in the minimum inhibitory concentration. Several other studies were conducted to evaluate the efficacy of both natural and synthetic EPIs on MDR strains of *K. pneumoniae* and other clinically significant nosocomial pathogens and proved it efficacious in inducing sensitivity toward various classes of drugs.[28,38-60] Related results were obtained where PAßN and reserpine tested against commonly used antimicrobial agents in the case of clinical isolates of *Enterobacteriaceae*.[61,62] Van Acker and Coenye[63] reported the role of PAßN, NMP, CCCP, and thioridazine in significantly reducing the biofilm formation in *E. coli*, *K. pneumoniae*, *Pseudomonas aeruginosa*, *Burkholderia cenocepaci*, and *Salmonella*. Aghayan et al[64] studied the potential effects of beberine and palmatine, naturally derived EPIs on the MexAB-OprM efflux system of *P. aeruginosa* isolated from burn infections. Rodrigues et al[65] suggested the use of EPI as therapeutic adjuvants to increase the efficacy of the existing antituberculosis drugs and to accelerate the treatment efficacy. Lv et al[66] identified a novel RND multidrug–efflux pump gene cluster (TMexCD1-TOprJ1) in *K. pneumoniae* isolated from animal source. Similar efflux pump sequence was also found in *P. aeruginosa*, *E. coli*, and *Salmonella*, which threatens the possibility of global dissemination. EPIs against the novel efflux system could restore the antibiotic sensitivity by decreasing the MIC of the drugs. Grimsey et al[67]demonstrated the efficacy of chlorpromazine and amitriptyline as EPIs to restore the antibacterial activity of the given antibiotics and inhibit the AcrB- mediated efflux. Abbas et al[68] investigated the potential inhibition activity of novel EPI (metformin) on AcrAB and Mdtk efflux pumps of *K. pneumoniae*. Their reports suggest the use of metformin over the

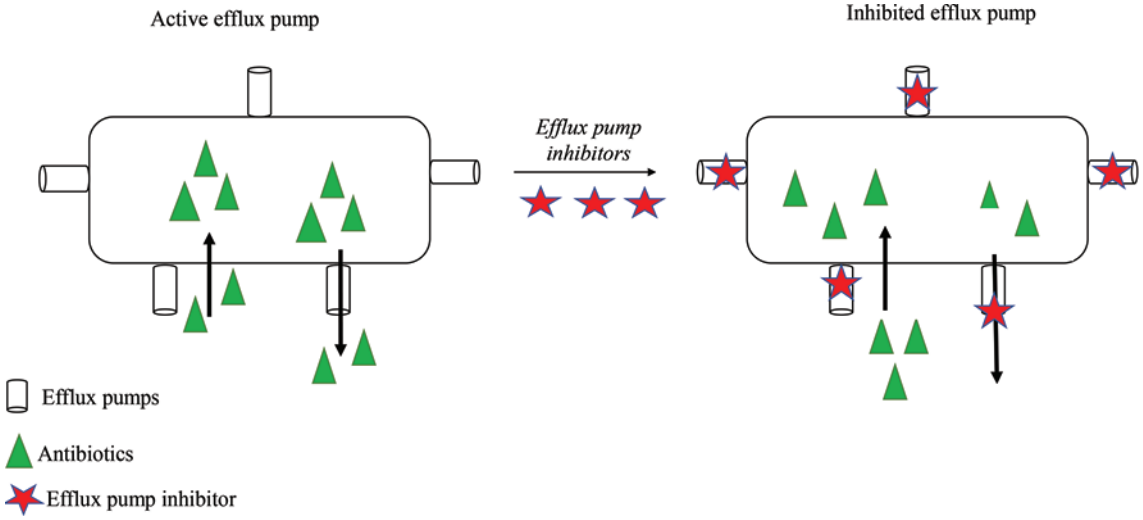

Fig. 2 Representation of mechanism of efflux pump inhibitor (EPI). (Modified from source Shriram et al. 2018).

existing EPIs ascorbic acid and verapamil in restoring the efficacy of the antibiotics. Reza et al[69] reported several EPIs as effective biofilm disruptors in the ESKAPE pathogens. Seukep et al[70] listed the use of numerous plant-derived secondary metabolites as nontoxic source of EPIs that would allow the use of antibiotics that were clinically ineffective due to resistance. Adam et al[71] developed six new classes of EPIs effective against *E. coli* AcrAB-TolC, clinical strains of *A. baumannii*, and *K. pneumoniae.*

Nevertheless, efflux pumps are a major resistant determinant in the increasing issue of bacterial infections worldwide. Identification of small-molecule EPI in restoring the effectiveness of the available antibiotics has restored hope. The greatest challenge in the generation of active EPI against Gram-negative bacteria, is that the penetration is controlled by porins, favorable to only zwitterionic and smaller hydrophobic molecules. On the other hand, major RND efflux pumps prefer large hydrophobic molecules.[72] In this instance, penetration into the bacteria could be improved by identification of suitable position on the EPI, by rational design by which the charge groups could be attached enhancing the EPI penetration. A group of pyranopyridine derivatives has been clearly shown to exhibit inhibitory effects against the AcrAB-TolC efflux system to date. Likewise, a series of pyridopyrimidinones were proved best against MexAB-OprM. D13–9001 was a well-optimized candidate which served effectively against *P. aeruginosa* efflux system (MexAB-OprM), but could not continue in clinical trials because it failed to inhibit MexXY-OprM.[72] The PAßN-derived EPIs are currently the most studied and developed families of inhibitors against *P. aeruginosa*.[72] To date, pyranopyridines, peptidomimetics, and indole derivatives have been patented as promising compounds. No EPIs have been clinically approved to date, mainly because of poor pharmacokinetic properties and low in vivo efficacy.[72]

Antisense Oligonucleotides

There is always a quest for an alternative therapeutic strategy in combating drug resistance conferred by efflux pumps in drug-resistant pathogens. In the quest, the antisense therapeutics contribute as an alternative antibiotic therapy with the aid of short single-stranded nucleic acid sequences modified to form stable oligomers. The molecules are termed antisense oligonucleotides (ASOs) due to their sequence complementarity to their messenger RNA (mRNA). ASOs modify the gene expression in a sequence-specific manner by binding to its complementary mRNA and thus inhibit its translation into protein.[72] Zamenick and Stephenson, pioneers in the field of antisense therapy, reported in Rou's sarcoma virus in infected chicken embryo fibroblast cells the addition of a 13-mer oligonucleotide complementary to the repeated sequences located at the ends of the genome inhibited their replication.[73] These scientists set a benchmark for the utilization of the antisense technology. Ever since this pioneering experiment was put forth, various strategies were designed as therapeutics effects for the treatment of diverse diseases like bacterial, viral, genetic disorders, and cancer. Toxicity, inability to penetrate the target, and nonspecific effects were the major challenges during the early years of antisense research which slowed down the progress. Following which, several antisense drugs of diverse chemical nature working through different mechanisms, including the utilization of siRNAs have been approved for trails.

ASOs could be exploited to suppress the bacterial antibiotic resistance determinants and increase its susceptibility. Increased antibiotic-resistant bacterial pathogens not only affect the ability to treat infectious diseases and complicate the medical procedure but also impose an economic burden on the health care system. ASOs technology permits the generation of antisense drugs that either have antibiotic activity or help to restore the ability of certain antimicrobials. Several studies have demonstrated the therapeutic efficacy and applications of the ASOs as mechanisms of inhibition to design therapies for bacterial infections.[74-83] A similar strategy of ASOs could be exploited against the MDR efflux system of *K. pneumoniae*. Versatility and high specificity of complementarity of the antisense oligonucleotides allow the generation of antisense drugs that disable resistance to antimicrobials and act as adjuvants to join the armamentarium to fight multidrug resistance.

This technology holds hope in the treatment of a diverse group of diseases including bacterial, viral, genetic disorder, and cancer. In the years to come we could witness the antisense adjuvants become a reality.

Conclusion

Bacterial resistance to antibiotics is a critical issue and efflux systems are influential in this phenomenon. Efflux systems are authoritative for intrinsic resistance in several pathogens. As they are located on the plasmids, they could be acquired by other microbes. Diverse efflux systems have been characterized, but the MDR transporters of the RND and MFS superfamilies are the most implied in the clinical resistance of Gram-negative bacteria. EPIs for such systems have been identified. Although selectivity and stability are the concerned drawbacks the results are yet encouraging to prospect in this field. Future works could consider improvising the inhibitory activity of the molecules. The antisense oligonucleotide is a promising alternative strategy with potential therapeutic applications in the field of medicine and industrial microbiology.

Conflict of Interest
None declared.

References

1 Afolabi OT, Onipede AO, Omotayo SK, et al. Hospital-acquired infection in Obafemi Awolowo university teaching hospital, Ile-Ife, southwest, Nigeria: a ten-year review (2000–2009) SLJBR 2011;3:110–115
2 Siu LK, Yeh KM, Lin JC, Fung CP, Chang FY. *Klebsiella pneumoniae* liver abscess: a new invasive syndrome. Lancet Infect Dis 2012;12(11):881–887

3 Donnenberg MS, Mandell GL, Bennett JE, Dolin R, *Enterobacteriaceae*In Principles and Practice of Infectious Diseases Pensilvania, PA: Elsevier; 2005 2567–2586
4 Podschun R, Ullmann U. *Klebsiella* spp. as nosocomial pathogens: epidemiology, taxonomy, typing methods, and pathogenicity factors. Clin Microbiol Rev 1998;11(4):589–603
5 Navon-Venezia S, Kondratyeva K, Carattoli A. *Klebsiella pneumoniae*: a major worldwide source and shuttle for antibiotic resistance. FEMS Microbiol Rev 2017;41(3):252–275
6 Priyanka A, Akshatha K, Deekshit VK, Prarthana J, Akhila DS, *Klebsiella pneumoniae* infections and antimicrobial drug resistance. In: Model Organisms for Microbial Pathogenesis, Biofilm Formation and Antimicrobial Drug Discovery. Singapore: Springer; 2020 195–225
7 Hsieh PF, Lu YR, Lin TL, Lai LY, Wang JT. *Klebsiella pneumoniae* type VI secretion system contributes to bacterial competition, cell invasion, type-1 fimbriae expression, and in vivo colonization. J Infect Dis 2019;219(4):637–647
8 Dunn SJ, Connor C, McNally A. The evolution and transmission of multi-drug resistant *Escherichia coli* and *Klebsiella pneumoniae*: the complexity of clones and plasmids. Curr Opin Microbiol 2019;51:51–56
9 Zhang L, Ma X, Luo L, et al. The prevalence and characterization of extended-spectrum β-lactamase-and carbapenemase-producing bacteria from hospital sewage, treated effluents and receiving rivers. Int J Environ Res Public Health 2020;174:1183–1196
10 Hersh AL, Newland JG, Beekmann SE, Polgreen PM, Gilbert DN. Unmet medical need in infectious diseases. Clin Infect Dis 2012;54(11):1677–1678
11 Boucher HW, Talbot GH, Bradley JS, et al. Bad bugs, no drugs: no ESKAPE! An update from the Infectious Diseases Society of America. Clin Infect Dis 2009;48(1):1–12
12 Solano-Gálvez SG, Valencia-Segrove MF, Prado MJ, Boucieguez AB, Álvarez-Hernández DA, Vázquez-López R, Mechanisms of resistance to quinolones. In: Antimicrobial Resistance IntechOpen; 2020. Published July 3, 2020. DOI: 10.5772/intechopen.92577
13 Padmini N, Ajilda AA, Sivakumar N, Selvakumar G. Extended spectrum β-lactamase producing *Escherichia coli* and *Klebsiella pneumoniae*: critical tools for antibiotic resistance pattern. J Basic Microbiol 2017;57(6):460–470
14 Mims C, Dockrell HM, Goering RV, Roitt I, Wakelin D, Zuckerman M. Attacking the enemy. Antimicrobial Agents and Chemotherapy: Macrolides.Medical Microbiology, 3rd ed. London, UK: Mosby Ltd. In: 2004;:489
15 Thomson JM, Bonomo RA. The threat of antibiotic resistance in Gram-negative pathogenic bacteria: β-lactams in peril! Curr Opin Microbiol 2005;8(5):518–524
16 Bennett PM. Plasmid encoded antibiotic resistance: acquisition and transfer of antibiotic resistance genes in bacteria. Br J Pharmacol 2008;153(Suppl 1) :S347–S357
17 Hawkey PM. Molecular epidemiology of clinically significant antibiotic resistance genes. Br J Pharmacol 2008;153(Suppl 1): S406–S413
18 Daikos GL, Kosmidis C, Tassios PT, et al. Enterobacteriaceae bloodstream infections: presence of integrons, risk factors, and outcome. Antimicrob Agents Chemother 2007;51(7): 2366–2372
19 Blanquart F, Lehtinen S, Lipsitch M, Fraser C. The evolution of antibiotic resistance in a structured host population. J R Soc Interface 2018;15(143):20180040
20 Zhou J, Dong Y, Zhao X, et al. Selection of antibiotic-resistant bacterial mutants: allelic diversity among fluoroquinolone-resistant mutations. J Infect Dis 2000;182(2):517–525
21 Gniadkowski M. Evolution of extended-spectrum β-lactamases by mutation. Clin Microbiol Infect 2008;14(Suppl 1):11–32
22 Davies J, Davies D. Origins and evolution of antibiotic resistance. Microbiol Mol Biol Rev 2010;74(3):417–433
23 Piddock LJ. Clinically relevant chromosomally encoded multidrug resistance efflux pumps in bacteria. Clin Microbiol Rev 2006;19(2):382–402
24 Coudeyras S, Nakusi L, Charbonnel N, Forestier C. A tripartite efflux pump involved in gastrointestinal colonization by *Klebsiella pneumoniae* confers a tolerance response to inorganic acid. Infect Immun 2008;76(10):4633–4641
25 Ogawa W, Minato Y, Dodan H, Onishi M, Tsuchiya T, Kuroda T. Characterization of MATE-type multidrug efflux pumps from *Klebsiella pneumoniae* MGH78578. PLoS One 2015;10(3):e0121619
26 Neuberger A, Du D, Luisi BF. Structure and mechanism of bacterial tripartite efflux pumps. Res Microbiol 2018;169(7-8):401–413
27 Padilla E, Llobet E, Doménech-Sánchez A, Martínez-Martínez L, Bengoechea JA, Albertí S. *Klebsiella pneumoniae* AcrAB efflux pump contributes to antimicrobial resistance and virulence. Antimicrob Agents Chemother 2010;54(1):177–183
28 Lamut A, Peterlin Mašič L, Kikelj D, Tomašič T. Efflux pump inhibitors of clinically relevant multidrug resistant bacteria. Med Res Rev 2019;39(6):2460–2504
29 Buckley AM, Webber MA, Cooles S, et al. The AcrAB-TolC efflux system of *Salmonella enterica* serovar *Typhimurium* plays a role in pathogenesis. Cell Microbiol 2006;8(5):847–856
30 Veleba M, Higgins PG, Gonzalez G, Seifert H, Schneiders T. Characterization of RarA, a novel AraC family multidrug resistance regulator in *Klebsiella pneumoniae*. Antimicrob Agents Chemother 2012;56(8):4450–4458
31 Blanco P, Hernando-Amado S, Reales-Calderon JA, et al. Bacterial multidrug efflux pumps: much more than antibiotic resistance determinants. Microorganisms 2016;4(1):14
32 Farhat N, Ali A, Bonomo RA, Khan AU. Efflux pumps as interventions to control infection caused by drug-resistance bacteria. Drug Discov Today 2020;25(12):2307–2316
33 Wong MH, Chan EW, Chen S. Evolution and dissemination of OqxAB-like efflux pumps, an emerging quinolone resistance determinant among members of Enterobacteriaceae. Antimicrob Agents Chemother 2015;59(6):3290–3297
34 Sharma A, Gupta VK, Pathania R. Efflux pump inhibitors for bacterial pathogens: from bench to bedside. Indian J Med Res 2019;149(2):129–145
35 Maurya N, Jangra M, Tambat R, Nandanwar H. Alliance of efflux pumps with β-lactamases in multidrug-resistant *Klebsiella pneumoniae* isolates. Microb Drug Resist 2019;25(8):1155–1163
36 Shriram V, Khare T, Bhagwat R, Shukla R, Kumar V. Inhibiting bacterial drug efflux pumps via phyto-therapeutics to combat threatening antimicrobial resistance. Front Microbiol 2018;9:2990
37 Fang CT, Chen HC, Chuang YP, Chang SC, Wang JT. Cloning of a cation efflux pump gene associated with chlorhexidine resistance in *Klebsiella pneumoniae*. Antimicrob Agents Chemother 2002;46(6):2024–2028
38 Türkel İ, Yıldırım T, Yazgan B, Bilgin M, Başbulut E. Relationship between antibiotic resistance, efflux pumps, and biofilm formation in extended-spectrum β-lactamase producing *Klebsiella pneumoniae*. J Chemother 2018;30(6-8):354–363
39 He F, Fu Y, Chen Q, et al. Tigecycline susceptibility and the role of efflux pumps in tigecycline resistance in KPC-producing *Klebsiella pneumoniae*. PLoS One 2015;10(3):e0119064
40 Hasdemir UO, Chevalier J, Nordmann P, Pagès JM. Detection and prevalence of active drug efflux mechanism in various multidrug-resistant *Klebsiella pneumoniae* strains from Turkey. J Clin Microbiol 2004;42(6):2701–2706

41 Kriengkauykiat J, Porter E, Lomovskaya O, Wong-Beringer A. Use of an efflux pump inhibitor to determine the prevalence of efflux pump-mediated fluoroquinolone resistance and multidrug resistance in *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 2005;49(2):565–570

42 Marrer E, Schad K, Satoh AT, Page MG, Johnson MM, Piddock LJ. Involvement of the putative ATP-dependent efflux proteins PatA and PatB in fluoroquinolone resistance of a multidrug-resistant mutant of *Streptococcus pneumoniae*. Antimicrob Agents Chemother 2006;50(2):685–693

43 Peleg AY, Adams J, Paterson DL. Tigecycline efflux as a mechanism for nonsusceptibility in *Acinetobacter baumannii*. Antimicrob Agents Chemother 2007;51(6):2065–2069

44 Garvey MI, Piddock LJ. The efflux pump inhibitor reserpine selects multidrug-resistant *Streptococcus pneumoniae* strains that overexpress the ABC transporters PatA and PatB. Antimicrob Agents Chemother 2008;52(5):1677–1685

45 Vidal-Aroca F, Meng A, Minz T, Page MG, Dreier J. Use of resazurin to detect mefloquine as an efflux-pump inhibitor in Pseudomonas aeruginosa and *Escherichia coli*. J Microbiol Methods 2009;79(2):232–237

46 Zhang Z, Liu ZQ, Zheng PY, Tang FA, Yang PC. Influence of efflux pump inhibitors on the multidrug resistance of *Helicobacter pylori*. World J Gastroenterol 2010;16(10):1279–1284

47 Fankam AG, Kuete V, Voukeng IK, Kuiate JR, Pages JM. Antibacterial activities of selected Cameroonian spices and their synergistic effects with antibiotics against multidrug-resistant phenotypes. BMC Complement Altern Med 2011;11:1–1

48 Srinivasan VB, Vaidyanathan V, Mondal A, Rajamohan G. Role of the two component signal transduction system CpxAR in conferring cefepime and chloramphenicol resistance in *Klebsiella pneumoniae* NTUH-K2044. PLoS One 2012;7(4):e33777

49 Zhong HQ, Zhang S, Pan H, Cai T. Influence of induced ciprofloxacin resistance on efflux pump activity of *Klebsiella pneumoniae*. J Zhejiang Univ Sci B 2013;14(9):837–843

50 Magesh H, Kumar A, Alam A, et al. Identification of natural compounds which inhibit biofilm formation in clinical isolates of *Klebsiella pneumoniae*. Indian J Exp Biol 2013;51(9):764–772

51 Srinivasan VB, Rajamohan G. KpnEF, a new member of the *Klebsiella pneumoniae* cell envelope stress response regulon, is an SMR-type efflux pump involved in broad-spectrum antimicrobial resistance. Antimicrob Agents Chemother 2013;57(9):4449–4462

52 Venter H, Mowla R, Ohene-Agyei T, Ma S. RND-type drug efflux pumps from Gram-negative bacteria: molecular mechanism and inhibition. Front Microbiol 2015;6:377

53 Dolatabadi A, Noorbazargan H, Khayam N, et al. Ecofriendly biomolecule-capped Bifidobacterium bifidum-manufactured silver nanoparticles and efflux pump genes expression alteration in *Klebsiella pneumoniae*. Microb Drug Resist 2021;27(2):247–257

54 Kuete V, Ngameni B, Tangmouo JG, et al. Efflux pumps are involved in the defense of Gram-negative bacteria against the natural products isobavachalcone and diospyrone. Antimicrob Agents Chemother 2010;54(5):1749–1752

55 Srinivasan VB, Singh BB, Priyadarshi N, Chauhan NK, Rajamohan G. Role of novel multidrug efflux pump involved in drug resistance in *Klebsiella pneumoniae*. PLoS One 2014;9(5):e96288

56 Zheng JX, Lin ZW, Sun X, et al. Overexpression of OqxAB and MacAB efflux pumps contributes to eravacycline resistance and heteroresistance in clinical isolates of *Klebsiella pneumoniae*. Emerg Microbes Infect 2018;7(1):139–150

57 Laudy AE, Mrowka A, Krajewska J, Tyski S. The influence of efflux pump inhibitors on the activity of non-antibiotic NSAIDS against gram-negative rods. PLoS One 2016;11(1):e0147131

58 Krishnamoorthy G, Leus IV, Weeks JW, Wolloscheck D, Rybenkov VV, Zgurskaya HI. Synergy between active efflux and outer membrane diffusion defines rules of antibiotic permeation into Gram-negative bacteria. MBio 2017;8(5):e01172–17

59 Cramariuc O, Rog T, Javanainen M, Monticelli L, Polishchuk AV, Vattulainen I. Mechanism for translocation of fluoroquinolones across lipid membranes. Biochim Biophys Acta 2012;1818(11):2563–2571

60 Iman Islamieh D, Afshar D, Yousefi M, Esmaeili D. Efflux pump inhibitors derived from natural sources as novel antibacterial agents against *Pseudomonas aeruginosa*: a review. Int J Med Rev 2018;5:94–105

61 Khan F, Pham DTN, Oloketuyi SF, Manivasagan P, Oh J, Kim YM. Chitosan and their derivatives: antibiofilm drugs against pathogenic bacteria. Colloids Surf B Biointerfaces 2020;185:110627

62 Xu Q, Jiang J, Zhu Z, et al. Efflux pumps AcrAB and OqxAB contribute to nitrofurantoin resistance in an uropathogenic *Klebsiella pneumoniae* isolate. Int J Antimicrob Agents 2019;54(2):223–227

63 Van Acker H, Coenye T. The role of efflux and physiological adaptation in biofilm tolerance and resistance. J Biol Chem 2016;291(24):12565–12572

64 Aghayan SS, Kalalian Mogadam H, Fazli M, et al. The effects of berberine and palmatine on efflux pumps inhibition with different gene patterns in *Pseudomonas aeruginosa* isolated from burn infections. Avicenna J Med Biotechnol 2017;9(1):2–7

65 Rodrigues L, Cravo P, Viveiros M. Efflux pump inhibitors as a promising adjunct therapy against drug resistant tuberculosis: a new strategy to revisit mycobacterial targets and repurpose old drugs. Expert Rev Anti Infect Ther 2020;18(8):741–757

66 Lv L, Wan M, Wang C, et al. Emergence of a plasmid-encoded resistance-nodulation-division efflux pump conferring resistance to multiple drugs, including tigecycline, in *Klebsiella pneumoniae*. Mbio 2020;11(2):e02930-19

67 Grimsey EM, Fais C, Marshall RL, et al. Chlorpromazine and amitriptyline are substrates and inhibitors of the acrb multidrug efflux pump. MBio 2020;11(3):11

68 Abbas H, Shaker G, Khattab R, Askoura M. A new role of metformin as an efflux pump inhibitor in *Klebsiella pneumoniae*. J Microbiol Biotechnol Food Sci. Accessed June 30, 2021 at https://doi.org/10.15414/jmbfs.4232 Published April 7, 2021. Accessed …

69 Reza A, Sutton JM, Rahman KM. Effectiveness of efflux pump inhibitors as biofilm disruptors and resistance breakers in gram-negative (ESKAPEE) bacteria. Antibiotics (Basel) 2019;8(4):229

70 Seukep AJ, Kuete V, Nahar L, Sarker SD, Guo M. Plant-derived secondary metabolites as the main source of efflux pump inhibitors and methods for identification. J Pharm Anal 2020;10(4):277–290

71 Green AT, Moniruzzaman M, Cooper CJ, et al. Discovery of multidrug efflux pump inhibitors with a novel chemical scaffold. Biochim Biophys Acta, Gen Subj 2020;1864(6):129546

72 Hegarty JP, Stewart DB Sr. Advances in therapeutic bacterial antisense biotechnology. Appl Microbiol Biotechnol 2018;102(3):1055–1065

73 Marwick C. First “antisense” drug will treat CMV retinitis. JAMA 1998;280(10):871

74 Yanagihara K, Tashiro M, Fukuda Y, et al. Effects of short interfering RNA against methicillin-resistant *Staphylococcus aureus* coagulase in vitro and in vivo. J Antimicrob Chemother 2006;57(1):122–126

75 Fooladi AA, Aghelimansour A, Nourani MR. Evaluation of the pathogenesis of *Pseudomonas aeruginosa*’s flagellum before and after flagellar gene knockdown by small interfering RNAs (siRNA) Jundishapur J Microbiol 2012;6

76 Hillman T. Antisense inhibition of *acc*A in *Escherichia coli* suppressed *lux*S expression and increased antibiotic susceptibility. BioRxiv 2020:747980./prpt

77 Suzuki Y, Ishimoto T, Fujita S, et al. Antimicrobial antisense RNA delivery to F-pili producing multidrug-resistant bacteria via a genetically engineered bacteriophage. Biochem Biophys Res Commun 2020;530(3):533–540
78 Wu S, Liu Y, Zhang H, Lei L. Nano-graphene oxide with antisense vicR RNA reduced exopolysaccharide synthesis and biofilm aggregation for *Streptococcus mutans*. Dent Mater J 2020;39(2):278–286
79 Verma D, Negi A, Pant K, Pant B, Thapliyal A. Designing of short interfering RNAs (siRNAs) for tuberculosis. IJITEE 2020;3:3022–3027
80 Mohanty PS, Sharma S, Naaz F, Kumar D, Raikwar A, Patil SA. Inhibition of *Mycobacterium tuberculosis* tRNA-ligases using siRNA-based gene silencing method: a computational approach. J Comput Biol 2020;27(1):91–99
81 Gong FY, Zhang DY, Zhang JG, et al. siRNA-mediated gene silencing of MexB from the MexA-MexB-OprM efflux pump in *Pseudomonas aeruginosa*. BMB Rep 2014;47(4):203–208
82 Patil SD, Sharma R, Srivastava S, Navani NK, Pathania R. Downregulation of yidC in *Escherichia coli* by antisense RNA expression results in sensitization to antibacterial essential oils eugenol and carvacrol. PLoS One 2013;8(3):e57370
83 Meng J, Kanzaki G, Meas D, et al. A genome-wide inducible phenotypic screen identifies antisense RNA constructs silencing *Escherichia coli* essential genes. FEMS Microbiol Lett 2012;329(1):45–53