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Efflux Pumps In Multidrug Drug resistant- *Klebsiella pneumoniae*: Discovery of Efflux Inhibitors

Sapna Joshi, Akanksha Chaudhary, Ranjeet Kumar, Rajeev Singh Kushwaha, Deepika Biswas

[1]Department of Biochemistry, Combined PG Institute of Medical Sciences and Research, Kuanwala, Dehradun, India
[2]Government Doon Medical College, Dehradun, Uttarakhand, India

Corresponding Authors Dr. Deepika Biswas, biswas.deepika89@gmail.com

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ABSTRACT

Klebsiella pneumoniae is a gram negative, that causes several health implications like inflammation in lungs (bacterial pneumonia), meningitis, UTI, intra-abdominal infection etc. The aim of current study is to depict the multidrug resistance induced by various classes of efflux pumps attributed to *K.pneumoniae* strains and the bacteria accommodates profuse number of efflux pumps like RND, SMR, ABC, MFS etc. It has the ability to export a broad range of toxic compounds into the external environment from within the bacterial cell, including many kinds of antimicrobial drugs. This study also gives brief description on efflux pump inhibitors like reserpine, phenylalanine-arginine β-naphthylamide (PAßN), flavonoids, piperine, carbonyl cyanide-chlorophenylhydrazone (CCCP), etc. These pumps are capable of extruding a wide variety of antibiotics, and they are often enhanced due to mutations in regulatory genes such as ramR and acrR. Targeting these pumps with EPIs has the potential to refine the efficiency of accessible antibiotics and address the global challenge of antibiotic resistance faced by this powerful disease. Nevertheless, efflux pump inhibitors (EPIs), which may be found naturally or artificially, have shown promise as potential therapies for K. pneumoniae, a bacteria that is resistant to several drugs.

INTRODUCTION

Klebsiella pneumoniae causes nosocomial infection, is an opportunistic pathogen, gram negative, facultative anaerobic, non-motile, oxidase negative, catalase positive, and typically

encapsulated bacteria (1,2). Plasmids and mobile genetic elements allow horizontal gene transfer (HGT). It is principally responsible for the development of AMR in these organisms (2). There are hundreds of studied mobile AMR genes subjected to HGT among the bacteria that are Gram negative (3). In part to the synthesis of plasmid-encoded AmpC Cephalosporinases and Ambler class A extended-spectrum-lactamases (ESBLs), Klebsiella pneumoniae has developed resistance to expanded-spectrum cephalosporins (4). Mrk proteins are encoded by the mrkABCDF operon and are mannose-resistant *Klebsiella*-like (Mrk) hemagglutinins, are essential for the development of *K. pneumonia* biofilms (5). By removing toxins or antimicrobial agents, the efflux pumps of bacteria regulate the internal environment. Bacterial efflux pumps are transmembrane proteins that are present throughout all bacteria (6). The widespread multidrug efflux pump named Acriflavine resistance B (AcrB) in *Klebsiella pneumoniae* is the pre-eminent multidrug efflux system (7).

They play a critical role in multidrug resistance because they may export a wide range of harmful chemicals and many distinct types of antimicrobial drugs from within the bacterial cell into the external environment (6, 8).

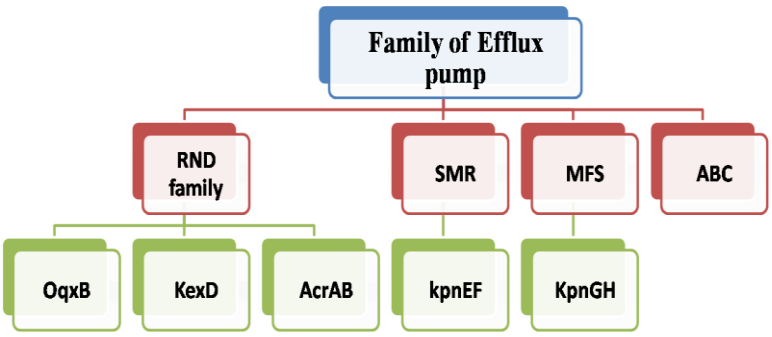

Fig.1.Efflux Pumps of *Klebsiella pneumoniae*

Efflux pumps: classification and clinical significance in *K.pneumoniae*

Transmembrane proteins known as bacterial efflux pumps are found throughout all bacteria and are able to export a diversity of toxic substance, together with several diverse classes of

antimicrobial agents, into the surrounding environment. Efflux systems involved in antimicrobial resistance include ATP-binding cassette (ABC), Resistance-nodulation-division (RND), Small multiantibiotic resistance (SMR), Major facilitator superfamily (MFS), and (9).

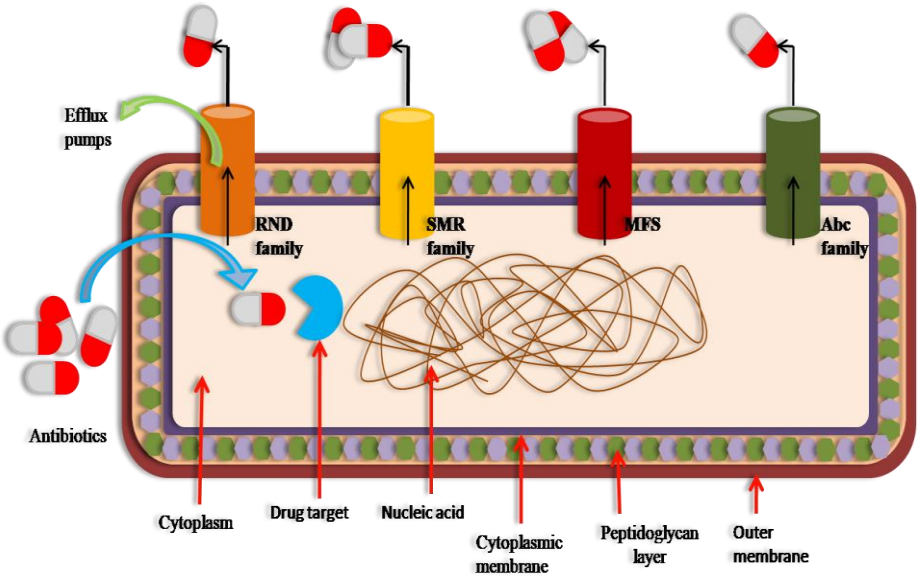

Figure 2. Schematic diagram of Efflux pumps in *Klebsiella*

A. RND (Resistance-Nodulation-Division) type efflux pumps

It has been shown that overexpression of the intrinsically encoded RND-type efflux pumps AcrAB and OqxAB, which is caused by mutations in the transcriptional regulator genes ramR and acrR, is a critical component of tigecycline resistance in K. pneumoniae. B-lactams are extruded by a number of RND family pumps, and they prevent the production of peptidoglycan in the extracellular periplasmic region. The RND-type efflux pumps are made up of the periplasmic protein, the outer membrane channel, and the RND inner membrane protein.

These three components are thought to work together to create a tripartite efflux pump that crosses both the inner and outer membranes (10). We cloned each gene deduced from K. pneumoniae MGH78578 encoding RND-type efflux pumps downstream of the lac promoter to assess the substrate specificity of each RND-type pump and the drug-resistance pattern in

the isolated resistant mutants. The plasmids were then used to transform *E. coli* (KAM32 or KAM33) and *K. pneumoniae* ATCC 10031. The *E. coli* strains, (KAM32 and KAM33), which are TG1 derivatives; do not contain acrB and acrAB, respectively. Next, the minimum inhibitory concentrations (MICs) of antimicrobial agents, antiseptics, and antibiotics in these transformants were ascertained. It was decided that kexC or kexD required the host's periplasmic component, AcrA, in order to operate, thus a plasmid carrying them was inserted into KAM32. As was predicted, AcrAB, OqxAB, and EefAB had shown higher levels of resistance to several chemicals. KexEF also shown extremely high resistance to a range of compounds, including AcrAB, OqxAB, and EefAB (11).

- **OqxB efflux-** The crystal structure of OqxB was purified and solved at 1.85 Å resolution. The trimer's overall fold resembles that of other RND class efflux pumps from the HAE1 subfamily that have been reported, such as AcrB and MexB. Each OqxB monomer is made up of 12 trans-membrane (TM) helices (TM1 to TM12). Superposition of the OqxB monomer onto the asymmetric AcrB trimer revealed that it exhibited 2.42 Å and 2.71 Å RMSD with the access/loose and extrusion/open conformations of AcrB, respectively, and that it is similar to the AcrB binding/tight monomer conformation with 1.86 main chain atoms RMSD. (12).
- **KexD efflux pump-** AdeB from *Acinetobacter baumannii* and KexD were discovered to be substantially similar. Upstream of adeB is a gene named adeA, which is expected to encode a periplasmic protein, and downstream of adeB is a gene called adeC, which is expected to encode an outer membrane protein. The upstream and downstream areas bordering kexD, however, did not contain any genes that encode periplasmic. Due to the absence of any such genes in the area around the kexD locus, it was hypothesized that KexD had both an outer membrane component and a periplasmic component that it had acquired from another source.

B. AcrAB efflux pump- The AcrAB multidrug efflux system is encoded by the K. pneumoniae acrRAB operon. The AcrAB repressor is encoded by the operon acrR, whereas the integral membrane protein with 12 membrane-spanning helices and the 40 kDa periplasmic lipoprotein tethered to the inner membrane that joins the outer and inner membranes are encoded by acrA and acrB, respectively (13). Schneiders et al. found that in 19 fluoroquinolone-resistant K. pneumoniae strains, increased synthesis of the AcrAB efflux pump was caused by either mutations in the AcrAB repressor, AcrR, or overexpression of the transcriptional regulator RamA. (14).

C. SMR (Small multidrug resistance) Type Efflux pumps : The smallest efflux proteins of the SMR family, are about 107 amino acid residues long. These multidrug transporters work as homo-oligomeric complexes because of their tiny size (15). The four-transmembrane (TM)-stranded -helical proteins produced by SMR proteins are distinguished by the short length amino acid (100 to 150 AA) (5). Three subclasses of the SMR protein are the small multidrug proteins, suppressor of groEL mutations, and paired small MDR (16).

- **kpnEF-like family** protein used in the production of capsules, which may increase *K. pneumoniae* pathogenicity. Greater sensitivity to hyperosmotic (2.8-fold) and high biliary (4.0-fold) concentrations was seen in the kpnEF mutant. Increased susceptibility to cefepime, ceftriaxone, colistin, erythromycin, rifampin, tetracycline, and streptomycin was caused by mutations in kpnEF; mutant strains changed from resistant to susceptible after complementation, and the resistance was regained. (16).

D. MFS (Major facilitator family) Type Efflux pumps

Membrane transport proteins known as MFS pumps can uniport, antiport, or symport a wide range of substrates, such as antibiotics, sugars, oligosaccharides, phosphate esters, and intermediates of the Krebs cycle (15). In Gram-negative bacteria, a subset of inner membrane

proteins in the MFS work as efflux pumps to reduce intracellular concentrations of certain dangerous substrates and to confer multidrug resistance (17). The second-largest group of membrane proteins is the MFS efflux pumps, that are proton dependent transporters involved in the efflux of just a few substrates. EmrD, the first MFS efflux pump discovered in *E. coli*, was studied as a potential uncoupler of oxidative phosphorylation by disruption of the proton gradient (6).

- **KpnGH-** substances like deoxycholate, acriflavine, EtBr, and SDS. KpnGH showed sensitivity to triclosan, chlorhexidine, and benzalkonium chloride (17).

E. ABC (ATP-binding casette) Type Efflux pumps

The ABC transporters, the biggest family of transporter proteins, are distinct from other families in that they obtain their energy from ATP hydrolysis (6). ABC-type ATPases are most frequently referred to as ABC transporters. They are present in many biological systems, including those of humans and bacteria, and are unquestionably crucial to many cellular processes because they carry important nutrients into or out of a particular type of cell (18).

Potential therapeutics targeting efflux pumps in MDR *Klebsiella pneumoniae*

1. Modes of Efflux pump inhibitord (EPIs)

EPIs may bind on same site as substrates and operate as competitive inhibitors, obstruct the appropriate binding of substrates through a noncompetitive mechanism, and act by impeding the proper action of efflux pump (19). When standard antibiotics are no longer working, inhibiting these efflux pumps may seem like a good plan of action. The development of natural substrates and efflux pump inhibitors (EPIs) that allow for efficient accumulation of drug inside of bacterial cells improves antibacterial efficacy. Reserpine, piperine, flavonoids, and geraniol are some of the plant-derived efflux pump inhibitors in use (20).

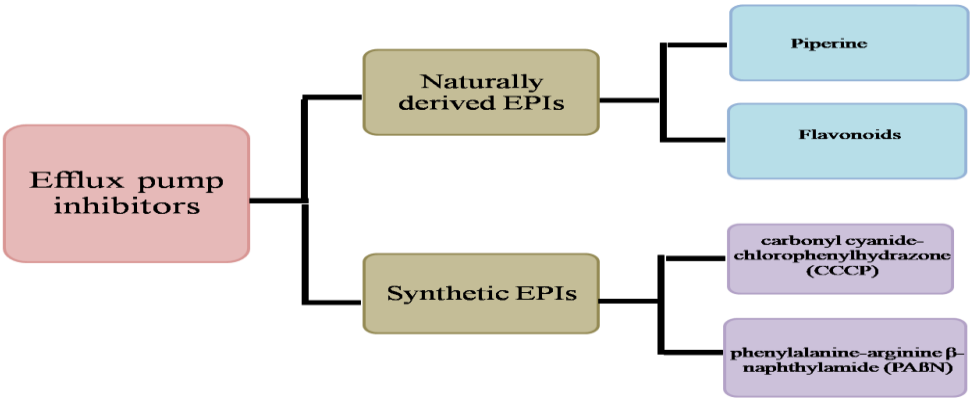

Fig.3.Flow chart of Efflux Pump Inhibitors

1.1. Naturally derived EPIs

- **Reserpine**- By inhibiting the vesicular monoamine transporters, it works by permanently preventing the uptake/storage of dopamine into synaptic vesicles. The efflux pumps of RND superfamily, MFS, and membrane protein classes are the targets of the potential efflux pump inhibitors. Reserpine is said to enhance medication therapy by directly interact with the amino acids in the EPT, which is a hallmark of few efflux proteins (21).
- **Piperine**-The pharmacological potentiator piperine prevents human P-glycoprotein from functioning, (22), especially those mediated by cytochrome P450, and it affects animal models' ability to suppress glucuronidation activity. Especially those mediated by cytochrome P450, and it affects animal models' ability to suppress glucuronidation activity. Meanwhile, there is an increase in some drugs' bioavailability and some treatments' effectiveness (23).
- **Flavonoids-** A number of phytochemicals are considered to be potential sources of novel EPIs because they are substrates of efflux pumps in the defence against bacterial infection, such as terpene and flavonoids (24).

1.2. Synthetic EPIs

1.2.1. Carbonyl cyanide-chlorophenylhydrazone (CCCP) -The primary role of Carbonyl cyanide-chlorophenylhydrazone (CCCP) is to interfere with the proton motive force of membranes, acting as an uncoupler for oxidative phosphorylation in the process. Gram-negative bacteria with colistin resistance can have their resistance inhibited and reversed by CCCP. CCCP has inherent cytotoxicity, hence it cannot be used in the clinic directly (25).

1.2.2. Phenylalanine-arginine β-naphthylamide (PAßN) -When used at low concentration (26.3 mg/liter), the diamine compound phenylalanine arginine -naphthylamide (PAβN) can boost the effectiveness of the antibiotics tetracycline, chloramphenicol, sparfloxacin and norfloxacin against different entero-bacterial isolates that over-produce efflux pumps such as AcrAB-TolC (26). The test results showed that strains of *E. aerogenes* and *K. pneumoniae* have a PAβN-susceptible efflux mechanism, as shown by the significantly lower MICs for fluoroquinolones and chloramphenicol in the presence of PAβN. When the PAβN addition caused threefold reduction in the MIC of an antibiotic compound, efflux pump activity was discovered (27). Although PAβN (EPI) can increase intracellular ATP levels, it can't overcome additional resistance mechanisms present in clinical isolates, for instance a target mutation or else altered permeability (28).

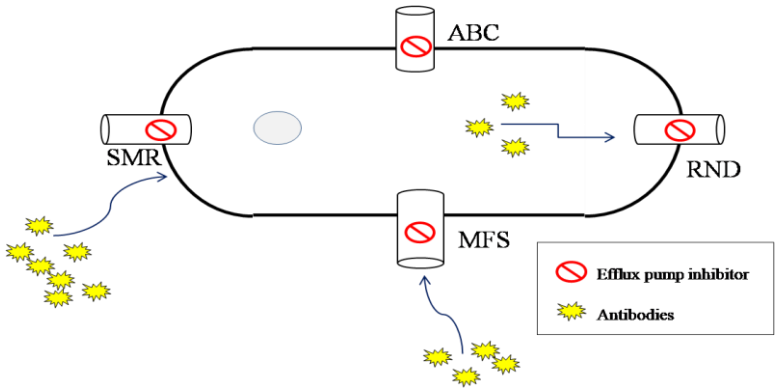

Fig.4. Schematic diagram of Efflux Pump inhibition in *Klebsiella pneumoniae*

Conclusion:

Due to its multidrug resistance, *K. pneumoniae*, a powerful opportunistic pathogen, poses a huge global concern. Efflux pumps, which are important in the expulsion of a variety of antimicrobial drugs, have become a well-known resistance determinant in this bacterium. RND, SMR, MFS, and ABC types of efflux pumps are included in the classification of efflux pumps, as well as each type contribute to the development of MDR through a different mechanism. AcrAB, OqxAB, and EefAB are examples of RND-type efflux pumps, which are important in *Klebsiella pneumoniae* resistance. These pumps, which are frequently amplified as a consequence of mutations in regulatory genes like ramR and acrR, be able to extrude a wide range of antibiotics. Additionally, it has been determined that *K. pneumoniae's* OqxB efflux pump contributes to the emergence of antibiotic resistance.

Resistance be also facilitated by SMR-type efflux pumps, such as KpnEF, which are particularly effective against colistin and aminoglycosides. When overexpressed, these tiny multidrug transporters work as homo-oligomeric complexes and are linked to enhanced resistance.KpnGH is one of the MFS-type efflux pumps that contributes to multidrug resistance and lower intracellular concentrations of dangerous substrates. These pumps are proton-dependent transporters that help remove different substrates from the body.

ABC-type efflux pumps deliver critical nutrients while using ATP hydrolysis as their energy source. Although less is known about their function in *K. pneumoniae* resistance, this field of study is still crucial.

Both synthetic and naturally occurring efflux pump inhibitors (EPIs) have demonstrated promise as possible treatments against multidrug-resistant *K. pneumoniae*. These EPIs can boost medication absorption within of bacterial cells, enhancing the effectiveness of antibiotics. EPIs that have been studied for their capacity to inhibit include reserpine, piperine, flavonoids, CCCP, and PAβN. In conclusion, it is critical to comprehend the classification and clinical significance of efflux pumps in *K. pneumoniae* when coming up

with plans to fight infections that are resistant to a variety of drugs. Targeting these pumps with EPIs has the potential to develop the efficiency of existing antibiotics along with address the global challenge of antibiotic resistance faced by this powerful disease. The discovery of novel medicines to deal with this urgent public health concern depends on more study in this area.

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Conflicts of Interest
The authors declare that there are no conflicts of interest.

Ethical statement
The authors declare that no experiment involves animals or humans.

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