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Role of nanomaterials in deactivating multiple drug resistance efflux pumps – A review

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ABSTRACT

The changes in lifestyle and living conditions have affected not only humans but also microorganisms. As man invents new drugs and therapies, pathogens alter themselves to survive and thrive. Multiple drug resistance (MDR) is the talk of the town for decades now. Many generations of medications have been termed useless as MDR rises among the infectious population. The surge in nanotechnology has brought a new hope in reducing this aspect of resistance in pathogens. It has been observed in several laboratory-based studies that the use of nanoparticles had a synergistic effect on the antibiotic being administered to the pathogen; several resistant strains scummed to the stress created by the nanoparticles and became susceptible to the drug. The major cause of resistance to date is the efflux system, which makes the latest generation of antibiotics ineffective without reaching the target site. If species-specific nanomaterials are used to control the activity of efflux pumps, it could revolutionize the field of medicine and make the previous generation resistant medications active once again. Therefore, the current study was devised to assess and review nanoparticles' role on efflux systems and discuss how specialized particles can be designed towards an infectious host's particular drug ejection systems.

1. Introduction

Nanotechnology has found its way into almost all research fields due to its particular dimension, and surface-to-volume ratios harbor unique features of great potentials (Prabhu and Poulose, 2012b). Nanotechnology has been employed to control bacterial growth and resistance to a great extent in microbiology (Chatterjee et al., 2014). The growing crisis of increasing resistance to various antibiotics is a significant concern that the world faces today (Ayukekbong et al., 2017). Multidrug-resistant organisms (MDROs) are the most life-threatening (Boucher et al., 2009). They are the prime reason behind morbidity and fatality in all age groups (Ismail et al., 2018). For the formulation and development of recent generation antibiotics, enormous amounts of labor and funding are required (Spengler et al., 2017). Table 1 depicts the dosage-based resistance pattern in pathogens (Badar and Khan, 2020). Even bringing a new drug into the market after all the trials is time-consuming. As pathogens are exposed to increasing concentrations of drugs, they tend to become tolerant to the specific drug (Hassan et al., 2015a). Their cellular mechanism is equipped to find alternate strategies to make these static and cidal drugs as tolerable toxins (Ramirez et al., 2013). Hence, it's always appreciated to find ways to avoid the tendency of the organism to gain resistance. Table 2 represents the average rate of resistance prevalent among World Health Organization (WHO) priority pathogens towards the corresponding antibiotics in India. There has

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Table 1
Comprehensive dosage-based resistance pattern in pathogens (Badar and Khan, 2020).

Medication	Dosage (mg)	Organisms
Ampicillin	10	*K. pneumoniae, S. epidermidis, S. maltophilia*
Amikacin		*A. baumanii*
Cefepime	30	*S. epidermidis, Enterobacteriaceae*
Cefotaxime	30	*S. epidermidis, E. coli*
Ceftazidime	30	*S. epidermidis*
Cefuroxime	30	*K. pneumoniae, S. epidermidis*
Ciprofloxacin	5	*K. pneumoniae, A. baumanii*
Gentamicin	10	*K. pneumoniae, A. baumanii, S. maltophilia*
Imipenem	10	*A. baumanii, S. maltophilia*
Meropenem	10	*A. baumanii, S. maltophilia*
Methicillin	–	*S. aureus*
Trimethoprim	5	*S. maltophilia*
Levofloxacin	5	*A. baumanii*

Table 2
Average rate of resistance prevalent among World Health Organization (WHO) priority pathogens towards the corresponding antibiotics in India (Mogasale et al., 2021).

Organisms	Average Rate of resistance prevalent in India in 2019
E. coli	Carbapenem-resistant 45%, 3rd generation cephalosporin-resistance 54%
Serratia spp.	Carbapenem-resistant 90%, 3rd generation cephalosporin-resistance 20%
Shigella species	Fluoroquinolone-resistant 75%
Klebsiella spp	Carbapenem-resistant 54%, 3rd generation cephalosporin-resistance 63%
Salmonella species	Vancomycin-resistant 75%
Staphylococcus aureus	Methicillin-resistant *Staphylococcus aureus* (MRSA) 52%, Vancomycin-resistant 1%
Streptococcus pneumoniae	Penicillin-resistant 33%
Enterococcus faecium	Vancomycin-resistant 13%
Enterobacter spp	Carbapenem-resistant 57%, 3rd generation cephalosporin-resistance 7.2%
Proteus spp	Carbapenem-resistant 6.7%
Providencia spp	Carbapenem-resistant 25%
Others spp.	8.4%–92.9% for *Escherichia coli* and from 4.1% to 79.4% for *Klebsiella pneumoniae*

been a drastic increase in resistance patterns among pathogens in the past five years (Mogasale et al., 2021). Nanoparticles against MDROs have provided ways and mechanisms of mitigating the issue of drug resistance in a very innovative manner (Baptista et al., 2018; HuhandY and Kwon, 2011). Particles in the nano-range have been successfully utilized in microbiology and the pharmaceutical industry for their great therapeutic applications.

Nevertheless, each cellular mechanism of resistance is affected by a different function of the antibiotics. Therefore, it is essential to learn about the common drug-resistant strategies used by the bacteria and how they can be hindered or altered. Among the various techniques available or opted, efflux is a unique system that is made to flush out any unnecessary and toxic substance out of the cell (Sun et al., 2014). These are generally membrane-bound proteins with sites for various substrates to specifically bind on them and activate the target substance's exclusion process. They also fall under the category of transporter proteins. They are known to have an immense drug pumping out the property (Nikaido and Pagès, 2012). All the diseases related to the pathogens having an efflux system tend to give rise to strains resistant to multiple drugs. Sometimes they are extensively drug-resistant. However, when these resistant drugs are administered in conjugation with nanoparticles, they have been reported to be very effective.

Many metallic nanoparticles have an inherent antimicrobial activity to hinder these cellular systems of efflux or biofilms. Their combination with conventional drugs has shown synergistic effects with various medications. The combined dosage of antibiotics and nanoparticles manifests superior antimicrobial effects compared to individual parent drugs (Ashajyothi et al., 2016; Christena et al., 2015; Fayaz et al., 2010; Gurunathan et al., 2014; Li et al., 2005). Due to their low toxicity (dosage dependent both *in vitro* and *in vivo*), these nanoparticles are preferred in association with broad-spectrum antibiotics. Their high surface area also adds to the overall antibacterial effect (Kulshrestha et al., 2017; Lewinski et al., 2008; Sintubin et al., 2012). It has also been noted that the combined dosage of the drug and metallic nanoparticles can lead to a decrease in the overall administration of medication and toxicity in patients (Kulshrestha et al., 2016). Several active researchers are toiling day and night to find out the exact mode of action of these nanoparticles, but no mechanism has been confirmed yet. In 2010, Banoee et al. (2010) came up with the mode of action of zinc oxide nanoparticles on efflux pumps. NorA pumps in *Staphylococcus aureus* were taken up for study against the nanoparticles. The zone of inhibition was found to increase for the ciprofloxacin antibiotic when used in combination with zinc oxide. The zone for ciprofloxacin increased by 22% for *Escherichia coli* and 27% for *Staphylococcus aureus* (Banoee et al., 2010).

Later Padwal et al. (2014), investigated the synergistic effect of iron oxide nanoparticles functionalized by polyacrylic acid in the presence of rifampicin antibiotic (PAA-MNP). The target bacterium under study was *Mycobacterium smegmatis*. When the combinational drug PAA-MNP has experimented, the zone of inhibition got increased by four times when compared to the zone produced by the antibiotic alone. The mode inferred by the scientist was that there was an increase in the intracellular concentration of the drug for the new combinational drug. A ubiquitous substrate for efflux pump (Ethidium bromide) was used to confirm the inferred mechanism in real-time (Padwal et al., 2015). When it comes to efflux pumps, there are two major mechanisms through which they can be hindered. The direct binding of the hindering molecule to the specific site of the pump on the cell membrane. This aids in blocking the drugs' flushing out of the cytosol and hence, killing the cell. They generally follow a competitive inhibition mechanism to interfere with a given specific efflux pump. Hence, the pump remains dormant and the drug stays inside for longer (Padwal et al., 2014). The second method of hindrance is an efflux system, which would interrupt the efflux kinetics. For example, MexAM-OPrM, a type of efflux pump in *P. aeruginosa* leading to multidrug resistance, can effectively be disrupted by silver nanoparticles. The efflux pump's kinetics are disturbed, and the bacterium becomes sensitive to drugs (Nallathamby et al., 2010). They first eliminate the proton gradient, leading to a fall of the membrane potential or reduction in PMF (proton motive force). Finally, the overall driving force required to initiate the efflux activity is terminated (Choi et al., 2008; Dibrov et al., 2002). The major point of concern in this step is that these nanoparticles are so small and reactive that they tend to bind with other membrane components, structures, and pumps. The interaction of nanoparticles with the efflux system exclusively is quite complex. In another study, it was reported that copper nanoparticles effectively inhibited norA pumps as they generated copper ions. However, the contribution of copper ions was partially responsible for the overall inhibition of the efflux pump. The major reason for inhibition was proposed to be the direct binding of copper nanoparticles to the pump. The copper ions aided in the disruption of the efflux kinetics and energy levels, therefore, hindering the activation of the efflux pump (Ashajyothi et al., 2016). When strains of *Escherichia coli* were grown in different concentrations of copper nanoparticles, they showed a reduction in the membrane potential up to −75 mV (Chatterjee et al., 2014).

The explicit role of nanoparticles in efflux inhibition and the associated mechanism is still unexplored. When it comes to translational particles in nano-range for healthcare and medicine, one should be very sure about the synthesis protocol followed, the physiochemical changes caused by the particles both *in vitro* and *in vivo*, the kinetics and dynamics have undertaken along with biodistribution in the body (Boucher et al., 2009). The universal phenomenon of multidrug resistance is seen in both gram-positive and negative strains. Each new drug

is potentially exposed to many mechanisms of resistance (Magiorakos et al., 2012). The microorganisms most commonly associated with MDR are *Staphylococcus aureus, Pseudomonas aeruginosa*, *Enterobacter* spp, *Enterococcus faecium*, *Acinetobacter baumannii,* and *Klebsiella pneumoniae*. For easy remembrance, they are made into an acronym represented as ESKAPE. The world health organization has declared these organisms a priority for researchers to develop medications and techniques to control their growth and virulence. Among the above-mentioned bacterial genera, three of them are termed as very critical organisms attaining MDR. These are *Enterobacter*, *Acinetobacter,* and *Pseudomonas* (Organization, 2017). Apart from the WHO-recommended genera, researchers have found sufficient MDR species such as *Shigella, Escherichia coli,* and *Proteus mirabilis*. The CDC (disease control and prevention organization) has reported that over 2 million people are affected due to ESKAPE every year. The mortality related to the same is also around 23,000 annually (Najafi, 2016). The feature of drug resistance is an outcome of selective pressure that the bacteria undergo. These selective pressures are developed due to illiteracy, lack of awareness, inadequate prescription practices, unauthorized and unsupervised sale of drugs, restricted and unsatisfactory diagnosis, ignorance on the knowledge of drugs and their modes of action, and finally, illegal use of medications (Ayukekbong et al., 2017).

Each antibiotic has its mode of action on the bacterial cell structure. Various components are targeted to kill the pathogen. They might be the genetic material or the protein production components or the cellular wall and membrane, or the organelles responsible for the overall metabolism of the bacteria. The drugs directly attacking the bacterial cell wall should be designed to bind to specific complementary receptors for initiating cell death. However, those drugs that work inside the cell should be tailored to have the ability to cross the cytoplasmic membrane and reach the target organelle for manifesting its antibacterial effects. An efflux pump is found to create a positive selective pressure for the MDR bacterial strains by letting them thrive in an environment with a reduced concentration of the drug (Masi et al., 2017). These membrane-bound organelles are found in all cells in both prokaryotes and eukaryotes. A source of energy that is mostly chemical in nature is required to activate these pumps and expel the drugs from the intracellular portion of the cell. Additional modes can also be associated with the active efflux pump mechanism, which can degrade the active drug through lysis or modification of the drug to bind falsely to other organelles, deviating from the target or tampering with the drug cell permeability to hinder the drug intake into the pathogen. Quorum sensing and biofilm formation also form alternate strategies combined with active efflux to gain slow and passive resistance (Spengler et al., 2017).

A very expressive and unique exclusion pump will allow the pathogen to sustain an extended range of compounds. Hence, they evolve into superbugs. Superbugs are known to be the cause of the resurgence of several virulent diseases worldwide. Innovative and smart solutions are on the way to tackle these superbugs. The imperative necessity to find novel approaches in medicine and drug design is pointed out by these superbugs. Newer and fresh modes of action combined with therapies and techniques from all disciplines are the need of the hour (Nikaido, 1994). In this review, various nanoparticles active against MDROs and their possible modes and strategies of antibacterial effects have been summarised. The kinetics and dynamics of combined drugs are also discussed.

2. Bacterial efflux and strategy

Before studying the effects of nanoparticles on the efflux pumps, it is ideal to know the structural and physiological elements of this transporter protein system. Alternative measures and treatments are gaining momentum to overcome the scarcity of new drugs to tackle these MDR strains. Use of nanoparticles, incorporation of herbal therapy is trending nowadays. The basic instinct of survival in pathogens has overshadowed the hope created by the dawn of antibiotics. Few microbes naturally tend to inhibit many first-generation drugs. However, recently, the ability of various previously susceptible microorganisms to gain resistance has caught the eyes of many researchers. The codons encoding for resistance in bacteria are either present in the plasmid, transposons, or the main chromosome. They are reported to be transmitted both horizontally and vertically (Kumar and Varela, 2013). The tendency of the pathogen to adapt effectively to adverse environments and a good capacity of genetic exchange among other strains has made MDR possible. Knowing the exact mechanism of these MDROs will aid in finding a breakthrough to eliminate these pathogens efficiently. Hierarchically, there are two significant types of resistance attained by the bacterial cells. First is natural resistance, also known as intrinsic resistance. This resistance is common to the strains belonging to the same species due to the structural and functional characteristics of the specific strain. The second is clinical resistance, also known as acquired resistance due to genetic mutation in the codons of the target organelles or components of the bacterial cell. The presence of an additional layer exterior to the cell membrane of mycobacteria and gram-negative bacteria results in restricted permeability of any foreign material from outside the cell. Hence, this hydrophobicity attributes to natural resistance (Nikaido, 1994). This structural feature in gram-negative bacteria throws light on the phenomenon of better resistance in the former than in the gram-positive strains. It is also termed as a passive form of resistance. Even efflux pump activation leads to the same effects. However, when associated with efflux pump, the process of resistance due to membrane permeability is considered as an active process (Poole, 2005). Bacterial strains generally modify the cellular targets to the corresponding antibiotic to gain resistance. Whatever the antibiotic's mode of action, each class or generation of antimicrobials has its own set of clinically resistant bacteria. Each resistance arises due to spontaneous mutations at the target site. Later, alterations take place under selective conditions to gain MDR. For example, resistance to rifamycin is possible due to mutation in RNA polymerase. The same can be said for quinolone resistance if the mutation occurs in DNA gyrase. Sometimes, conjugation, transformation, or transduction also attribute to resistance in many organisms. The relocation of the virulent gene from one strain to other is seen in most of the cases. For example, resistance to methicillin is reported to be due to the acquisition of the corresponding gene (mecA) in *Staphylococcus aureus* strains.

Similarly, the van gene encodes for glycopeptide resistance in *Enterococci* (Lambert, 2005). These genes can tend to overexpression of the pumps, which leads to hinder various antimicrobial effects of many classes of drugs. Along with the efflux pump inhibitors, membrane permeability and plasmid-based beta-lactamase inhibitors go hand in hand to reinforce resistance in pathogens. Duval and their team investigated *Listeria monocytogenes* for their mode of resistance. Generally, the protein synthesis components such as ribosomes are blocked. Still, this bacterium tended to split its protein-synthesizing ribosomes to enhance protein synthesis and increase the stability of the cell for survival (Duval et al., 2018). Several reports have shown various unexpected ways of instilling resistance in bacteria.

2.1. Efflux carriers

Active efflux is the prime mechanism used by bacterial cells to remove any unwanted waste from the cell. This also includes antimicrobial compounds given to restrict the growth of the microbe (Wright, 2011). The efflux system is an energy-based membrane transportation system. It contains sites for specific recognition of complementary molecules. The attachment across the binding site is also very apt.

Once the binding is over, transport across the membrane is initiated. An active receptor or binding site on the pump can be specific to a given drug or may allow multiple binding of similar and competitive components. When multiple binding and overexpression are feasible for an efflux pump, the characteristic trait of MDR is achieved by microbes (Liu

et al., 2013). If we go into basics, the membrane-bound transporter proteins are categorized into various families. These classifications are based on the cassette used for ATP binding (ABC), cell nodulation for resistance section (RND), extrusion of toxic and multidrug compounds (MATE), resistance to small multidrug (SMR) and superfamily facilitator (MFS). They are denoted as primary structural components of the efflux pump. Some species have additional carriers associated with their pumps. For instance, *A. baumannii* has a particular carrier for eliminating the antibacterial compounds of Proteobacterial origin. They are termed as PACE in short. These carriers are found to be having structural similarities close to the native efflux family SMR (Hassan et al., 2015b). Among the above reported 5 carrier families, ABC uses ATP to remove toxic substances from the pathogenic cell. They are the leading primary carrier in efflux pumps. At the same time, the other 4 carrier proteins use the electrochemical energy existing in the transmembrane. Proton motive force is preferred for RND, MFS, and SMR, while for MATE, sodium motive force (Nishino and Yamaguchi, 2001) is preferred. Based on the cell membrane composition, the number of pump carriers on the surface varies. Membranes with peptidoglycan layers mostly have singular efflux carriers. They can either be ABC or MFS or SMR. On the contrary, membranes with lipopolysaccharides have severe complexities. Various researchers report a combination of carriers including 600 amino acid alpha helical protein structure is the backbone of MFS carrier. They are arranged in a helical structure consisting of 12 or 14 moieties. The mode of passage through the carriers is seen to be either symport, uniport, or antiport in nature (Paulsen et al., 1996). The existence of an additional layer in gram-negative strains tends to make the efflux pump adapt to the tripartite structure. Several well-defined carrier pumps of MFS have been documented for MDR gram-positive strains. To name a few, they are QacB, MdfA NorA, QacA, KpnGH, LmrP, CraA, etc. These are present in *S. aureus, E. coli, K. pneumoniae, L. lactis,* and *A. baumannii,* respectively (Schindler and Kaatz, 2016). These proteins have successfully instilled resistance against tetracycline, chloramphenicol, streptomycin, norfloxacin, cefepime and ceftazidime. The smallest carrier in the lot is SMR. It consists of 100–120 amino acids arranged in membrane-bound helices (4 in number). This smallest carrier family is subdivided into three, namely, a paired protein, suppressor protein for mutation suppression, and small pumps for multidrug. This carrier is found to be so potent that even a single gene expression has the ability to induce multiple drug resistance in both gram-positive and negative bacteria (Bay et al., 2008; Roca et al., 2009; Srinivasan et al., 2014). In some strains such as *E. coli,* SMR has 110 amino acids. The SMR protein is here termed as EmrE protein, and it aids in the expulsion of ammonium and acriflavine compounds (Banigan et al., 2015). Some more examples to quote for SMR are EbrAB, QacC, and SepA. They belong to *B. subtilis* and *S. aureus*, respectively (Schindler and Kaatz, 2016).

RND proteins are a special class of carrier proteins that work devoid of the physiochemical trait of the toxic material. They are not altered by the amphiphilic or hydrophilic nature of the target substance. Hence, they are used for the exclusion of a broad range of molecules. They are comprised of 100 and more residues of amino acids. RND proteins associate with the outer, inner, and periplasmic spaces of the membrane due to their tripartite assembly. They are generally multimeric pumps. MexB and AcrB are proteins associated with the cytoplasm on the inner membrane. MexA and AcrA are periplasmic proteins that make up the body of the carrier protein. OprM and TolC are the proteins associated with the outer membrane. Hence, the overall structure of RND is consolidated. Some well-known RND carrier proteins are MexABOprM and AcrAB-TolC. They are found in *P. aeruginosa and E. coli*, respectively (Nishino and Yamaguchi, 2001). The ArcB range of carrier protein has an affinity to various medicines such as b-lactams, phenicol, fluoroquinolones, cyclin, and several solvents and detergents (Beketskaia et al., 2014). The unique structural setup of the whole RND prefers low molecular weight molecules (Takatsuka et al., 2010). It has been suggested previously through various hypotheses that each sub-class of RND undergoes periodical changes in its conformation. Complimentary alterations further influence these changes to the nearby units. Similarly, deactivation of any one of the subunits will lead to total loss of the entire RND protein function. This typically happens if there is a delay or problem in proton transmission in the subunits (Takatsuka and Nikaido, 2009).

Lastly, Cassette for ATP binding (ABC) is an energy-dependent primary carrier protein harbored in the trans-membrane region of the cell. It is found in all types of living cells (Davidson et al., 2008) and has an affinity to many target molecules. Its substrates can be sugars, peptides, metal ions, etc., and it is the largest protein carrier in the family of efflux pumps. The most significant ABC protein has around 1200 residues of amino acids. These large moieties are arranged systematically in 4 domains. Out of 4, two domains are hydrophobic. They aid in recognition of the target and then the transport out of the cell. These two domains are found deep in the trans-membrane space. The other two domains are exposed in the cytoplasmic region. They bind with ATP and break it down by hydrolyzing it (Schmitt and Tampé, 2002). In a strain with lipopolysaccharide layers, the primary carrier protein (ABC) is associated with the adapter protein in the periplasmic space and the protein on the cell's outer membrane. This interaction aids in letting the foreign molecules pass through the membrane and get expelled out into the extracellular portion of the cellular lining. An ideal example to quote is the well-known MacAB-TolC protein of *E. coli*. It is unidirectional in operation and ATP-driven (Gupta et al., 2011). MATE carrier protein causes hydrophilic medications inactive in most pathogens. They harbor approximately 450 residues of amino acids. They also have 12 structurally similar membrane segments. The most common medications that are made inactive due to this carrier efflux protein are aminoglycosides and fluoroquinolones (Putman et al., 2000). Most organisms have MDR because of the presence of MATE carrier protein that generally uses sodium motive force. However, some unique bacteria such as *A. baumannii, P. aeruginosa,* and *S. aureus* use proton motive force. The genes associated with these organisms would be AbeM, PmpM and MepA, respectively (Kaatz et al., 2005; Kuroda and Tsuchiya, 2009).

2.2. *Efflux and quorum sensing*

Efflux systems work hand in hand with other forms of mechanisms that cause resistance in bacterial species. They have a very vital role in signaling between cells and between molecules and cells. This leads to the formation of biofilms in pathogens. To effectively tackle therapeutic resistance in microbes, it is necessary to inhibit both efflux and quorum sensing systems of a virulent pathogen. This will result in restriction of biofilm formation. Several researchers have proposed the synergistic effect of efflux expulsion systems on quorum sensing (Bay et al., 2008; Hassan et al., 2015b; Nishino and Yamaguchi, 2001; Roca et al., 2009; Srinivasan et al., 2014). When resistance increases in a particular strain, it will by default increase the population of the concerned species in the given environment. As the efflux system keeps flushing contents into the surrounding environment, specific inducers are introduced into the extracellular region of the cellular population by the virulent strains. As bacterial cell communication is high-speed and simple, the other cells in that area adapt to the virulent pathogens' resistance. Hence, resistance is spread to the given population of bacteria. These autoinducers can accumulate in the surroundings and enhance population density, thereby leading to alterations in the genetic makeup of the organism. The genes associated with quorum sensing always trigger expression towards the population's phenotype surviving in proximity. Many functions are controlled and monitored by quorum sensing. These include antibiotic secretions, bioluminescence, production of spores, biofilms, etc. Even virulence initiating in bacteria is controlled by this phenomenon, as it manages the respective factors and proteins that cause resistance to drugs (Novick, 2008). Each strain of bacterium has its own mechanism of quorum sensing followed by unique components for regulation of its physiological functions. There are three basic guidelines

that every quorum sensing system follows. Initially, inducers are generated automatically from the bacterial cells. These will start cellular communication while signaling the neighboring species. When the cellular density in an area is low, the inducers will diffuse away from the parent cell and are present in low concentrations to be detected by any technique. As the population of the bacterial species increases, the combined and elevated production of inducers from each cell leads to a higher concentration of the same in a given localized area. This concentration is high enough to be assayed, and a suitable response is documented based on the scenario of the cells (Kaplan and Greenberg, 1985). The second step in the process is the reception of the inducers by suitable and specific receptors. These are located facing the cytoplasm or are anchored on the cell membrane. Finally, the genes associated with the cooperative environment between all the strains are activated by inducers (Kaplan and Greenberg, 1985). The only legit reasoning for the relation between efflux and quorum sensing is that the efflux system can aid in flushing the inducers and the toxic and undesirable wastes into the outside environment.

Nevertheless, a detailed relationship is still unknown and undocumented (Waters and Bassler, 2005). The inhibition of the efflux pump will hinder the concentration in the external environment and, hence, adversely affect quorum sensing (Xu, 2016). Suppose blockers for efflux systems are utilized effectively. In that case, the whole series of reactions and pathways are altered and will reduce undesirable film formation and reduce the transfer of resistance patterns between species. Even aggregation of neighboring cells is inhibited by the efflux pump's detrimental conditions (Xu, 2016). Metal-based nanoparticles have shown the tendency to restrict and hinder efflux systems in synergy with conventional antimicrobials. The ATP pathway for energy production is predominantly disrupted (Shah et al., 2006).

3. Alternate strategies for MDR treatment

Resistance in drugs has been reported for almost all the medications used for veterinary and human welfare. Therefore, it is essential to develop new ways and techniques to conserve the given resources of conventional medicine and make them as efficient as possible to face the current MDR crisis (González-Bello, 2017; Wright, 2016). Most of the drugs used are generally seen to be plant-based derivatives or chemical derivatives of the same. As of now, there are two exceptional drugs that can work on any new strain of bacterium. One is ACHN-975, and the other is Brilacidin. The former is a first-line emergency medication as an initial defense against most infectious species. The latter is still in clinical trials. The mode of action is seen to be LpxC inhibition. This enzyme is responsible for lipid A synthesis in pathogens. The current microbial and pharmaceutical research trend is to come up with the measures and components that can address the virulence/resistance issue of the microorganism and eliminate the bacteria as a whole. When a therapy or technique does not manipulate the growth and death of a microorganism but alters only the virulence, it is considered as an antipathogenic drug and will nullify the infectious nature of the pathogen. Therefore, the host doesn't get affected by the disease. The altering genes in the pathogenic strains can also generate antipathogenic effects. The severity of the disease and symptoms can be tailored easily to provide relief to the patients (Israil and Chifiriuc, 2009). Most of the efflux blockers also work towards the antipathogenicity of the organism. These compounds are much better sought for in comparison to designing new-generation drugs. Sometimes, they work towards breaking down the communication signals between the population of microbes. This induces antipathogenicity without being lethal to any cell. The outcome of this breach in communication between the cells would cause a reduction in selective pressure. This would lead to hindrance in the resistance of the pathogen (Waters and Bassler, 2005). It also manifests that the use of other techniques like gene manipulation also has a positive effect in controlling the virulence of a microorganism. Other techniques that might help are immune-based therapy and vaccine-based therapy. These techniques can also keep in check the feasible mutations that occur in the genetic makeup of an infectious agent. Therefore, controlling the resistance pattern in the pathogen (Lazar et al., 2005).

To have targeted delivery by nanomaterials, it is essential to know about the new site for the attack on an organism. This will aid in designing new medicines and innovative nanoparticles to target components that have enhanced antipathogenic and bactericidal effects. Before fabrication of nanoparticles for targeted delivery, it is essential to have a cumulative and detailed study of the pathogen and its environment using molecular biology, bioinformatics, proteomics, etc. This will generate a functional and knowledgeable database relating to the infectious agent and its modes of action, which in turn can pave the way for the required nanomaterial for treatment (Piddock et al., 2010). To gain information on the chemical aspects of the proteins and pathways associated with the virulence of the microorganism, chem-informatics can be opted. It provides a wide variety of data on ligand binding and its kinetics and relationship studies. It also throws light on the structural changes and conformational effects based on activity. These studies will provide a good screening of potential compounds similar to commercial drugs based on reasoning, logic, and experimental evidences (Hammami and Fliss, 2010). Similar research was performed in gram-negative strains by a group of scientists. They found an innovative aminoglycoside that was designed to target and inactivate its corresponding enzyme in the cell (Sardari and Dezfulian, 2007). Some minute and low molecular weight peptides with antimicrobial activity are also devised in labs for the same purpose. They can be extracted and tailored naturally or made synthetically too. Generally, their weight is kept to a maximum of 50 kDa. They are altered and created to attack cellular membranes and change the genetic makeup of the pathogen. They also interfere with the infectious agents' metabolic pathways, posing a suitable alternative treatment method for various infectious diseases (Ramirez et al., 2013). Nonetheless, the fabrication of these novel proteins is an expensive affair, significantly when escalated to scale up to commercialization processes. Their sensitivity to the host pathogen's proteolytic enzymes can lead to the peptide breakdown before reaching its target site. Their overall toxicity to the human body also needs to be considered before administering to patients. These peptides show enhancement in their features and properties when used in combination with nanotechnology. Then, nanosized or encapsulated forms can avoid degradation due to the host enzymes. Their availability and absorption are increased. The overall toxicity is also reduced significantly (Carmen Chifiriuc et al., 2014; Peters et al., 2010). However, these techniques need high investments and funding. They are also in need of several sophisticated equipments for fabrication and characterization. Hence, their applications are limited, and commercialization in the market becomes an issue.

4. Natural efflux pump inhibitors

Most of the efflux system blockers are in the nano-size range. Polyphenols of natural origin have always been a great source of efflux blocking compounds. They are stable in structure and chemically, very functional, and unique. It also harbors several metabolites of secondary origin with numerous pharmaceutical applications. Previously, many reports have been published on the use of herbal extracts in all organisms. Both gram-positive and negative species have successfully experimented. They also can restore the lost susceptibility to commercial antibiotics. The concentration of the drug inflow into the host is also elevated thereby, having an enhanced bactericidal effect. Few families are very much well known for their remarkable bioactive compounds as potential efflux system blockers of all the plant families available. A few potential plant families are *Zingiberaceae, Convolvulaceae, Apocynaceae, Lamiaceae, Berberidaceae, Fabaceae,* and *Cucurbitaceae*. Since being generally consumed as foods by the general crowd, their incorporation in medicines as efflux blockers is of growing interest. Many fruits, seeds, vegetables, and spices are investigated every day all around the world. A few active ingredients introduced in nanomaterials to enhance the efflux

systems are piperine, farnesol, ellagic acid, resveratrol, thymol, geraniol, p-coumaric acid, theobromine, etc.; their derivatives and metabolite forms are preferred in combination with nanomaterials. Among these are essential oils that are aromatic in nature. They possess a chemical gradient that has an affinity towards efflux blocking. Geraniol, carvacrol, farnesol, and thymol are well-known aromatic herbal plants with active components for prospective nanomaterial conjugation and utilization. They can inhibit the efflux system in 4 major modes. They either tackle the proton motive forces or the sodium motive forces. Sometimes, they act as competitive compounds for the binding sites on the efflux pumps, hence, keeping the pumps in an inactive state. Few molecules follow inhibition in a non-competitive way. Some other modes opted apart from these steps are disruption of the gradient across the membrane of the host. The factors associated with pathway regulation can also be hindered if the genes responsible for the same are blocked. Hence, the efflux expression decreases. The energy source ATP can be broken down or interfered by functionalized nanomaterials, which might increase the permeability of the liposaccharide layer. The drug crossing across the membrane increases, and the antibiotic effect is also enhanced. Direct change in the conformation of the pump is also feasible to get the required results to handle virulence in strains (Piddock et al., 2010).

5. Other sources

Apart from plant sources, several microorganisms can also produce compounds and by-products with similar activity towards the efflux pump (Stavri et al., 2007). *Streptomyces vellosus* produces specialized compounds such as EA-371 d, EA-371a, etc., that have a restriction effect on the efflux system of *P. aeruginosa* (Lee et al., 2001). Some strains have mutated down the years to produce novel metabolites to overcome the resistance attained by other strains. Termites extracted with alcohol produce metabolites that have antibiotic properties. They reduce the minimum inhibitory concentration of organisms like *E. coli and Staphylococcus aureus* (Coutinho et al., 2009). *Neonectria discophora* is refined and extracted to produce antimicrobial compounds to treat infections caused due to *Staphylococcus aureus* and *T. rubrum*. The active ingredients produced are derivatives of ilicicolinic acid (Chaves et al., 2015; Sorres et al., 2018). The process of extraction seems to be tedious for organism sources when compared to plant sources.

5.1. Metallic sources

Many metal-based nanoparticles play a crucial role in efflux inhibition. The most common metal-based nanoparticles are discussed in detail in the below sections.

Chitosan - This is a polysaccharide with a linear structure and linkages. It has a beta (1 → 4) linkage between its monomers. One deacetylated unit of glucosamine binds with an acetylated form of glucosamine. Chitosan is extracted from a natural polymer called chitin (Kumar, 2000). The special feature of this polymer is its compatibility with biological tissues and environment-friendly nature. It is potentially used as a drug carrier agent. Targeted and sustained release is feasible by this polymer. Drug temporin B has been successfully encapsulated in the nano-carriers of chitosan to enhance its antimicrobial activity (Piras et al., 2015). This polysaccharide in acidic environment attains a positive charge that could be of great harm to the oppositely charged genetic material. Even the cell membrane is negatively charged; hence, it has a great affinity to this polymer. When a detailed study was performed on gram negative strains for the effect of chitosan nanoparticles on them, it was found that these nanoparticles instilled a heightened affinity to the inhibitor. These are generally found in the growth media in the form of special dyes and micronutrients. There is an overall loss of the barrier function due to the affinity binding by the chitosan nanoparticles on the surface of the cell membrane. They cause extensive surface abrasions and cover the periphery as a sheath to block the vesicular functions (Helander et al., 2001). They also have shown evidence of binding with the cellular genetic material to hinder the essential transcription and translational processes (Pelgrift and Friedman, 2013). In 2009, the minimum inhibitory concentration of sulfamethoxazole was effectively reduced to a great extent when used along with chitosan in the nano range. The decrease was seen to be five folds. *Pseudomonas aeruginosa* has an over-expressive efflux system (MexEF-OprN). This carrier is affected the most by these chitosan nanoparticles (Tin et al., 2009). The suggested reasoning provided by the researchers is that the combinational synergy between the drug and chitosan nanoparticles attained a change in the expression of the efflux carriers.

Silver – this metallic compound has been in practice to treat various illnesses since ancient times. It is very much well known for its antimicrobial activity. Greeks in their ancient history had reported the use of nanocrystals to treat a broad spectrum of pathogenic species. Approximately 150 different strains of bacteria are effectively treated using silver nanoparticles (Mijnendonckx et al., 2013). Derma treatment for burns, soothing drops for eyes, and tending to chronic wounds have used silver for many centuries. Even trachoma has been avoided with great precision due to the utilization of this metal (Lemire et al., 2013). Catheters used in body bags have been lined with polymers that are conjugated with silver. The antimicrobial activity of the metal leads to inhibition of biofilm formation on the walls of the catheter. Even topical formulations for skin ailments contain silver to a greater extent. Most of the formulations of silver are generally in the nano range (Maillard and Hartemann, 2013; Prabhu and Poulose, 2012a). Although the microbial inhibition activity of silver is well experimented with and documented, the mechanism opted by the same is still unknown partially. Many researchers have inferred that the mode of action for silver is actually a combination of several mechanisms. One of the most common modes is the passage of metallic nanoparticles across the membrane. This will lead to the accumulation of the same in the intracellular compartments. The activation of the reactive oxygen species is triggered to produce free radicals. This leads to the shutting down of the essential enzymes, finally breaking down the genetic makeup of the host-pathogen. This also hampers the activation and working of efflux pumps (Prabhu and Poulose, 2012a). The ionic form of silver has a great affinity towards most moieties present in a cellular membrane. The thiol residues of the NADH bind with the silver ions along with the oxidoreductase of quinone. While binding, the structural integrity of the wall is brought down (Xu et al., 2004). This results in the block in the energy pathways of the pathogen, hence, adversely affecting the host bacteria. They nullify the DNA and RNA building blocks by the ROS mechanism. This hinders the central dogma of the cell. Even resulting protein and lipid conformations are damaged (Mijnendonckx et al., 2013). Two research teams have reported their novel synthesis of single silver metal nanoparticles. They were spherical and had surface plasmon resonance that was localized to a single spectrum of silver nanoparticles. Their experimental hosts were *Pseudomonas aeruginosa* and *Bacillus subtilis*. The effect of size on the kinetics of the ABC carrier of efflux pump was studied for both pathogens. The study was kept real time and used two very common instruments for characterization: a spectrophotometer and an optical microscope in dark field mode. The resolution was kept in nano range. They observed and inferred that the smaller the size of the nanoparticle the better the retention of the particle in the host-pathogen. The larger particles got easily flushed out with time. Therefore, they were able to give evidence to the fact that the size of the nanoparticle would influence the efflux pump kinetics, thus, affecting the resistance property of the bacteria (Lee et al., 2010). Similarly, size effect of nanoparticles and attainment of MDR in *Pseudomonas aeruginosa* were studied successfully in 2010. The transporter exclusively under study was the MexAB-OprM. When observed in nano-range resolution, the wild strains manifested higher retention of nanoparticles in the cells. The cells having the multidrug transporter in an overexpressed state showed a lower retention of the nanoparticles. When the transporter was blocked using an inhibitor, the results became favourable and reversed than previously

reported. Most commonly reported and used efflux blocker is CCCP (Carbonyl Cyanide m-Chlorophenylhydrazine), was also used as control in the experimental studies (Nallathamby et al., 2010).

The above strain of the bacterium was also studied by another group of scientists with aztreonam and chloramphenicol antibiotics. Three types of strains were studied for the same transporter. One being wild type, other being a mutant and overexpressing, known as nalB-1 and the last being a mutant that didn't express the gene MexAB- OprM. The latter was depicted as ΔABM. The controlling parameters and kinetics of the efflux carrier MexA-MexB- OprM were studied for both the mutants using both the drugs. In the absence of the aztreonam drug, the accumulation of nanoparticles was seen to be at a lower frequency inside the bacterium. As the time-based data were collected for the same, the number of retained nanoparticles didn't differ over the course of the experiment for nalB1. Instead, in ΔABM, the silver nanoparticle accumulation increased with time. When the aztreonam drug was administered, both the strains showed enhanced retention of nanoparticles over the course of the study. The increase was found to be very much proportional to time. When the study was closely monitored and inferred, it manifested that the carrier MexA-MexB-OprM played a vital role in retaining the nanomaterial in the cell. The synergy of the drug and the silver nanoparticles was very well depicted. The drug aided in bringing down the membrane integrity and the silver nanoparticles caused the toxicity as well as affected the efflux system of the host (Wijnhoven et al., 2009). Thus, the efflux system was summed under the pressure of multiple substrates (both silver nanoparticles and drug together). Similar results were seen for chloramphenicol also. The level and degree of accumulation in the intracellular space influenced the expression and kinetics of MexAB-OprM. The ΔABM mutant strain accumulated the largest number of silver nanoparticles in comparison to the nalB-1 strain. In the absence of the chloramphenicol drug, the accumulation and retention of nanoparticles were found to be good in both wild type and ΔABM. However, the nalB-1 had very low accumulations as their MexAB-OprM transporter was still functional and expressive. The rate of accumulation elevated up to 10 folds for a mere concentration of 25 μg/mL of the drug (chloramphenicol) in the wild and ΔABM mutant species. Nonetheless, in nalB, it didn't change at all. The nanoparticles accumulate on the cell membrane of the bacterial strain and does permanent damage to the structural conformation of the wall. This led to improper working of the efflux system (Xu et al., 2004). Hence, the resistance attained by any pathogenic species can be altered by silver nanoparticles in combination with the parent drug (Kyriacou et al., 2004; Li et al., 2005).

The synergistic effect of β-lactam drugs and silver nanoparticles were studied by Li et al. (2005)). They found that the combination of silver nanoparticles with drugs was found to be more lethal when compared to their individual effects. They hinder the respiratory pathway of the infectious host and destroy the permeability of the membrane (Shahverdi et al., 2007). This was found to be a parallel study to the above-mentioned studies. They worked with stronger medicines such as vancomycin, penicillin G, clindamycin, amoxicillin, erythromycin, etc. When used in conjugation with silver nanoparticles, their dosage of administration also decreased. The toxicity is easily monitored and controlled for the conjugated form of the drug (Mohammed et al., 2011). Proper evidences for cellular membrane and proton motive force damage have been reported via transmission electron microscopy and proteomic analysis (Li et al., 2011; Lok et al., 2007). In china, a group of scientists worked on the possible mechanisms of silver nanoparticles inside the cell. They inferred that the over accumulation of the nanoparticles led to condensation pressure on the chromosomes. The contents lost their ability to replicate and move freely into the surrounding environment. The host studied in this experiment was *Staphylococcus aureus*. The dehydrogenase enzyme that takes part in the respiratory and energy generation process is altered to have a reduction in its functional activity (Schairer et al., 2012).

Nitric oxide – recently the use of nitric oxide nanoparticles for bacterial growth and sustenance is of great interest to scientists. They seem to follow a wide range of modes to act against pathogenic strains. The primary mode would be to form reactive species of nitrogen oxide moieties that would interact and bind with many cellular proteins and cause disruption to the organelles. These reactive nitrogen oxide species have the tendency to attach with the DNA and RNA materials to initiate damages to the repair enzymes of the genetic materials. The respiration process of the pathogen is paused and affected as the reactive nitrogen oxide attaches to the zinc metalloproteinase enzyme (HuhandY and Kwon, 2011). This results in reduction in the efflux activity. Even small quantities of nitrogen oxide nanoparticles are found to be effective against some of the major MDR strains such as *Klebsiella pneumoniae*, methicillin resistant *Staphylococcus aureus*, *Enterobacter faecalis*, *Escherichia coli, Pseudomonas aeruginosa*, etc. The spectrum of action of nitrogen oxide species is very broad covering most of the pathogenic strains, be it gram positive or negative species. The membrane bound lipids and proteins are bound to alter the integrity of the plasma barrier, which in turn affects the efflux systems. However, the amount of virulence is noticed more in gram negative strains when compared to the gram positive strains (Raafat et al., 2008). Similar mechanism is noticed for nitric oxide nanoparticles on both gram positive and negative strains. They adhere to the components of the cell membrane and alter the integrity of the barrier. Hence, they are easily transported into the host.

Zinc – The mode of action of zinc nanoparticles is found to be almost similar to that of silver. They breakdown the membrane integrity and generate reactive oxygen species. These species will neutralize the genetic material of the bacteria and further tend to alter the conformations of lipids, proteins, etc. (Ghasemi and Jalal, 2016). Till date, zinc based nanoparticles are used in combinations with various commercial medicines to enhance their efficacy and overcome resistant strains at ease. Amoxicillin, erythromycin, cephalexin, cefotaxime, vancomycin, tetracycline, cloxacillin, gentamicin, clindamycin, ceftazidime, ampicillin, erythromycin, oxacillin, streptomycin, etc. show a great synergy when used in combination with zinc oxide nanoparticles (Venubabu Thati et al., 2010). Some have reported that based on the drug features and composition, the effect of nanoparticles varies based on the overall antimicrobial activity of the particle. Drugs such as penicillin G and amoxicillin manifested reduction in antibacterial activity against *Staphylococcus aureus*. However, ciprofloxacin, on contrary, had an increase in its antibacterial activity when used in combination with zinc oxide nanoparticles. The efflux system (NorA) falls short of energy as the whole pathway is disrupted by the nanoparticles. The highly resistant fluoroquinolones are made susceptible again via these nanoparticles (Couto et al., 2008). The membrane characteristic protein Omf also has a great affinity towards zinc oxide nanoparticles. They aid in persistent passage of quinolones through the membrane barrier. This leads to an enhanced absorption of the drug inside the cell (Banoee et al., 2010). Efflux setup is also hindered simultaneously. The formation of complexes between the drug and the zinc oxide nanoparticles also favours the abovementioned mechanisms. These were studied and documented in *A. baumannii* (Ghasemi and Jalal, 2016).

Gold- Aurum has its application in medicine since civilization. It has a very few effects on bacteria. When combined with potent drugs, it does wonders. Some medications used in combination with gold nanoparticles are mecapto ethylamine, 4, 6-Diamino-2 Pyrimidine thiol, 3-mercapto, p-Mercaptobenzoic acid, propyl sulfonate, etc. They tend to attach and form complexes with the transfer RNA and block the translational process of the subunits responsible for the efflux pump. Due to decrease in the structural units of the efflux pump, the whole system will collapse and resistance will be controlled. The ATP associated enzyme activity is also altered; hence, the energy supply goes down for the efflux pumps. *Enterococcus* species resistant to vancomycin, *E. coli* resistant to vancomycin and *Pseudomonas* species resistant to methicillin, etc. are treated with great care by gold nanoparticles (Hayden et al., 2012). When compared to other inert metal-based nanoparticles, gold is found to be very much superior in its design and applications. They are found

to be very much easy to be monitored when it comes to shape and size of the nanoparticle. Gold nanoparticles are functionalized easily using different ligands for different functions. They are stable and are biocompatible to living beings (Rosi et al., 2006). Gold is a very convenient platform used by numerous researchers to explore its potential in medicine. Its conjugation experimented with nucleotides, proteins, carbohydrates, single molecules, etc. is of great interest for scientists. These miniature moieties have potential biological applications and also, aid in designing fundamentals for the fabrication of specialized materials for diverse fields (Shahverdi et al., 2007). Particles that have multiple valences in the nano range can be used for surface modifications of gold. They are an ideal research model for testing polymer-based nanomaterials too. These remarkable gold nanoparticles are 100 times tinier than a general bacterium. When observed in solution, they exhibit a totally contrasting dimension when compared to other counterparts. Gold nanoparticles in conjugation with vancomycin have found to be elevating the bactericidal activity of the drug against its resistant strains such as Enterococcus. Their mode of action is depicted to be polyvalent inhibition of the efflux (Gu et al., 2003). N-acetyl lactosamine along with nanoparticles of gold has proven to be a good combo to restrict the attachment of enteropathogenic *E. coli* to the cell linings. Hence, their localizations are also hampered (Hyland et al., 2006). *Corynebacterium* is treated well with gold nanoparticles that create perforations in the cell wall of the pathogen. Later, they accumulate in the intracellular space and cause further damage to the efflux pump too (Mohamed et al., 2017). Gold nanoparticles functionalized by pyrimidine have a peculiar property of sequestrating the mineral ions such as magnesium and calcium present in the cell. This process results in damaging the bacterial wall and enhancement in permeation of the same. Even, the nucleic acids get transported out of the cell. Internalization of the nanoparticles into the cell leads to malfunctioning of the protein synthesis machinery that might affect the efflux pump system (Zhao et al., 2010). Therefore, the susceptibility to vancomycin increases in Enterococci when gold nanoparticles are used along with it. When the same conjugation is experimented with ampicillin drug it showed antimicrobial activity towards a broad spectrum of species. When ampicillin was used alone against most of the gram positive and negative strains, they were found to be resistant to the medication (Brown et al., 2012). The malfunctioning of the efflux system due to blocking by the gold nanoparticles and also, multivalent orientation of the drug to the pathogen are possible inferences that the researchers were able to point out. They were also seen to alter the tRNA of the translational pathway so that it could not bind to its required subunit and hence, collapsing the whole protein setup. Similar study was attempted by Khambesh and team and they received the same results for gold nanoparticles as reported above (Khameneh et al., 2016; Zhao et al., 2010).

Magnesium– the nanoparticle form of magnesium is generally its oxide form. Magnesium oxide nanoparticles generate reactive species of oxygen that tend to degrade the infectious host cell. However, few have reported that the magnesium oxide nanoparticles can have a reverse effect on the efflux system of the virulent strain (Pelgrift and Friedman, 2013). When the halogen group molecules are used in combination with magnesium oxide nanoparticles, the efficacy of the overall composite is enhanced to a great extent. Molecules of fluoride, chloride, bromide etc have a good affinity of adsorption on these oxide nanoparticles. Once reacted, they form their respective magnesium fluoride, magnesium chloride and magnesium bromide nanoparticles. Studies have reported that these compounds do have bactericidal effects against many pathogenic species such as *Staphylococcus aureus or Bacillus subtilis* (Hajipour et al., 2013).

Copper – Nanoparticles of copper are mostly preferred for electronics or material science purposes. Their utilization in efflux pump inhibitors and microbial control are quite scarce. Several researchers are now exploiting these nanoparticles for potential applications in MDR strain control. Many mechanisms and modes are proposed by various studies. Sometimes, they tend to directly bind to the bacterial membrane and cause damage. They also have the ability to produce free radicals that have an adverse effect on the cellular organelles of the host pathogen. Crosslinking of the copper nanoparticles with genetic materials such as RNA and DNA results in breakage and disorder in the whole structural setup of the chromosomes. Articles are also published on the conformational changes in the helical orientation of the DNA due to mutations by the nanoparticles. These mutations are seen to be either deletions or insertions that hinder the optimal expression of the codons. At times, these displace the vital metals associated with the cellular proteins and subunits (Christena et al., 2015). Few strains show a higher degree of susceptibility when compared to others. For example, *Pseudomonas aeruginosa* is not influenced much by copper nanoparticles but strains of *Staphylococcus aureus*, *Enterococcus faecalis* and *Escherichia coli* are greatly affected by the presence of copper nanoparticles (Bagchi et al., 2013). Several strains are concentration dependent for the bactericidal activity of the copper oxide nanoparticles. The minimum inhibitory concentration for each species varies with the different concentrations of nanoparticles administered. The efflux system of *Staphylococcus aureus* is found to be inhibited if a 0.032 mM of copper nanoparticles is introduced into the culture. However, bacterial pathogens such as *Pseudomonas aeruginosa* needs nearly double the concentration of the nanoparticles to hinder the efflux pump's functioning. When compared to conventional antibiotics, copper has better features as an antibacterial agent. The minimum inhibitory concentration of copper nanoparticles is reported to be far better than ciprofloxacin against methicillin resistant *Staphylococcus aureus* (Christena et al., 2015). The carrier associated with the resistance pattern in *Staphylococcus aureus* is the Nor A efflux carrier protein. These nanoparticles block them and make their functions inactive (Couto et al., 2008). In the same study, it was observed that at lower concentrations of the nanoparticles, copper aided the bacterial population to stop the production of biofilms and enhanced the sensitivity pattern in them (Christena et al., 2015). When lower dosages of copper nanoparticles are provided to the bacterial cultures, the regulators responsible for response to multiple antibiotics (Mar R) get changed. The structural moieties of MAR R have residues of cysteine in them. When copper comes in contact with them, they form tetramers by means of disulphide bonds. These tetramers stop the whole process of protein synthesis associated with biofilm formation. They also have shown a peculiar trait as a signalling agent in *Staphylococcus aureus*. This signalling alters the expression of the genes that are responsible for the external polysaccharide layers. The efflux pump blockers will also have a dual function of inhibiting biofilm formation too. Even films produced due to MFS and RND carriers can also be abolished with a help of a simple efflux blocker (Hao et al., 2014). The mediation in copper-based therapy is actually done by copper (II) ions. Only a part of its pharmaceutical activity is attributed by the nanoparticle form of copper.

Iron – Ferrous and ferric nanoparticles have several potential applications in the field of medical microbiology. They have the ability to retain and radiate heat. Hence, they are preferred for hyperthermia treatment on bacteria. They also are very suitable candidates for targeted drug delivery and therapy (Chertok et al., 2008). When it comes to imaging, it is a well-known image contrasting agent for MRI (magnetic resonance imaging) (Xing et al., 2013). The basic antibacterial action of iron nanoparticles is because of the oxidation of the intracellular components of the parent cell. The hydrogen peroxide generated in the pathways tends to give away hydroxide and ferrous ions. They are the stepping stones to the final reactive oxygen species that cause stress in the cells and disrupt the cellular components (Tran et al., 2010). Based on the presence of atmospheric oxygen, the effects of iron nanoparticles and their oxidative effects vary. Under anaerobic circumstances, the antibacterial activity is much more lethal and harsher than in aerobic or atmosphere saturated environments (Lee et al., 2008; Sies, 1997). The administration of functionalized iron nanoparticles with tuberculosis medications like rifampicin has aided in the treatment of TB resistant

strains of *M. smegmatis* (Padwal et al., 2014). They cause damage to the protein subunits and chromosomes of the bacterium. This might lead to malfunctioning of the efflux pumps. The only saving grace of theses nanoparticles when compared to other nanoparticles is the ease of fabrication and are inexpensive.

Calcium – this is a mineral that has its major applications in the field of implants and prosthesis. They cover dental and orthotic based aids and aesthetics. The calcium composite used in dentistry is made up of fillers in the form of glass for reinforcement in a matrix composed of polymer resin and finally, a coupling agent from silane. They have good mechanical and aesthetic properties, used in dental restoration and surgeries (Lim et al., 2002). When utilized for long, they have reported the formation of biofilms and plaque growth (Drummond, 2008). To increase the longevity of the implants, it is essential to load the matrix with bioactive agents to combat the microbial growth and resistance. Nanoparticles of calcium loaded with biologically active agents are used in dental implants to avoid recurrent infections, cavities and good mechanical load strength (Yoshida et al., 1999). Calcium nanoparticles can be paired with various antibacterial agents to reap several benefits. To name a few, the antibacterial agents associated with calcium nanoparticles are silver, quaternary ammonium salts, phosphates, etc. (Tanagawa et al., 1999). The phosphate forms of calcium release the ionic form of calcium along with phosphates in supersaturated concentrations. These ionic varieties help in repairing the cavities in the tooth and fill up the lesions. The combination of silver, quaternary ammonium salt in nano range and phosphates of calcium has shown restricted metabolic performance of the bacteria. The combo hinders colony accumulation near the teeth and film formation of *S. mutans*. The performance of the trio seems to be more promising when compared to two of the most commonly used commercial counterparts (Cheng et al., 2012). Some composites of calcium are pH dependent for their antibacterial activity. The hydroxide of calcium when used as dressing for intracranial lesions, manifested a potent bactericidal effect at alkaline pH around 12–13 (Byström et al., 1985). The presence of special active agents can also influence the performance of the calcium composites towards the bacteria. A group of scientists worked on the effect of calcium-based nanocomposite on *Enterococcus faecalis*. The effect of calcium hydroxide along with nanoparticles of chitosan with very minute amounts of functional disinfectant was seen on the efflux pumps of the same. There was no subsequent depletion in the film formation and even after 24 h, there was no breakdown on the film layers. The results didn't differ even when different concentrations of calcium hydroxide were administered. When disinfectant was employed in increased concentrations, they easily inactivated the 48 h old biofilm of *Enterococcus faecalis*. Introduction of additional inhibitor in the form of calcium hydroxide composite at minute concentrations, showed enhancement in the antimicrobial efficacy. At higher concentrations, this ability was lost (Upadya et al., 2011). However, many researchers don't find this calcium nanocomposite to be effective against *Enterococcus faecalis* for tooth related queries (Distel et al., 2002). The property of killing or hindering the growth of microbes is due to the calcium ions. In gram positive strains, their barrier membrane consists of carboxylate and phosphate moieties, which form binding sites for calcium ions (Hancock, 1997). There is no well documented study on the effect of calcium ions on efflux pumps. The ions of calcium are facilitated due to the hydroxide diffusion from the calcium hydroxide nanoparticles. The binding of the ions to the anionic moieties of the cell surface, neutralizes them and decreases the repulsion forces between them (Upadya et al., 2011). Therefore, it can be suggested that the nanoparticles of calcium act on the bacteria based on the ionic concentration of the same.

Platinum–Platinum is a precious metal that has been known to have adverse effects on many species of bacteria. Platinum nanoparticles have an affinity towards the protein subunits of the bacterial strains. They also have a preference to bind to the functional enzymes pertaining to replication and genetic material (Maye et al., 2005). The nanoparticles of platinum in colloidal forms have been reported to have anti-inflammatory effects on lungs and skin edema (Yoshihisa et al., 2010). They cause less harm to the environment when compared with its other metal counterparts. Hence, they are the preferred class of nanoparticles to mediate pathogens present in the natural environment (Rezaei-Zarchi et al., 2012). *Lactobacillus* species are found to be susceptible to platinum nanoparticles, functionalized by sucrose. Even *Pseudomonas stutzeri* is treated effectively using the abovementioned combination of platinum. These nanoparticles have their antibacterial effects based on their sizes. Very small nanoparticles of platinum ranging from 1 to 3 nm exhibited good antibacterial activity whereas an increase in size made them compatible to bacteria. A slight increase of 4–21 nm also made the platinum nanoparticles lose their bactericidal activity against *Pseudomonas aeruginosa* (Gopal et al., 2013). The exclusive effects of platinum nanoparticles on efflux pump are still under experimental stages. There are no concrete data for the same. Mostly, focus has been given on the combinatorial effects of platinum nanoparticles with structural building blocks of bacteria. Fig. 1 represents the graphical representation of the possible nanoparticles that aid in the deactivation of the efflux system.

Miscellaneous Nanoparticles - Aluminium nanoparticles were tested against efflux-based MDR *E. coli*. The size of the particles was in the range of 10–100 nm. Their main mode of action was found to be cell wall disruption (Lee et al., 2019; Zaidi et al., 2017). Similarly, titanium was used *against Staphylococcus aureus, Enterococcus faecalis,* and *E. coli.* Oxidation of the intracellular components were found to be their mode of action (Hemeg, 2017; Lee et al., 2019; Rudramurthy et al., 2016). In polymeric nanoparticles, lignin has been successfully used against efflux-based MDR *E. coli* and found to harbour genes encoding for membrane proteins with an efflux function were upregulated, others down-regulated (Hasani et al., 2019; Slavin et al., 2021).

6. Proposed mechanism of action

The bacterial strains with lipopolysaccharide layers have better structural efficacy in preventing the antimicrobial activity of nanoparticles. To be exact, the outer membrane is composed of porins as proteins, phosphate-associated lipids, and lipopolysaccharides (Couto et al., 2008). The other contemporary gram-positive bacteria has teichoic acids in them (Gupta et al., 2014). Although their compositions vary, ultimately, they maintain the negative charge on their exterior (Zechini and Versace, 2009). Teichoic acid is a linear polymer made up of phosphate moieties. Along with phosphate, amines and carboxyl group also add up to the negative charge of the gram-positive strains (Chevalier et al., 2010). The lipid A composition of the lipopolysaccharide contributes to the negative charge of the gram-negative species (Bohnert and Kern, 2005). Lipid A is actually a carbohydrate that is phosphorylated. The core is essentially an oligosaccharide (German et al., 2008).

Bacteria that have adapted themselves to the changing environment for survival pick up traits that help to survive—they stealth the drugs given against them. Even stimulus and disinfectants causing harm to the microbe are made ineffective by alterations made in the pathogen. This specialized population is denoted as persistent bacteria. The world bacterial population falling under this criterion is very less (around 1%). The very first resistant bacterial strain was reported in 1944. It was *Staphylococcus aureus* species (Imlay, 2013). Apart from creating trouble for the pharmaceutical industry to create new and advanced drugs on a regular basis, these species also have a key role in developing numerous long-time chronic conditions in patients (Imlay, 2015). The major mechanism by which nanoparticles tackle resistant bacteria is by creating stress. There is always a difference in the zeta potentials of the bacterium and the nanoparticles. Generally, bacteria will have around −26 mV, and the nanoparticles, for example, silver has around −15 mV. This difference in potential implies an attractive force between the pathogen and the nanoparticles. The outer envelope responds to the stress created by making the cell membrane of the bacteria positive. This

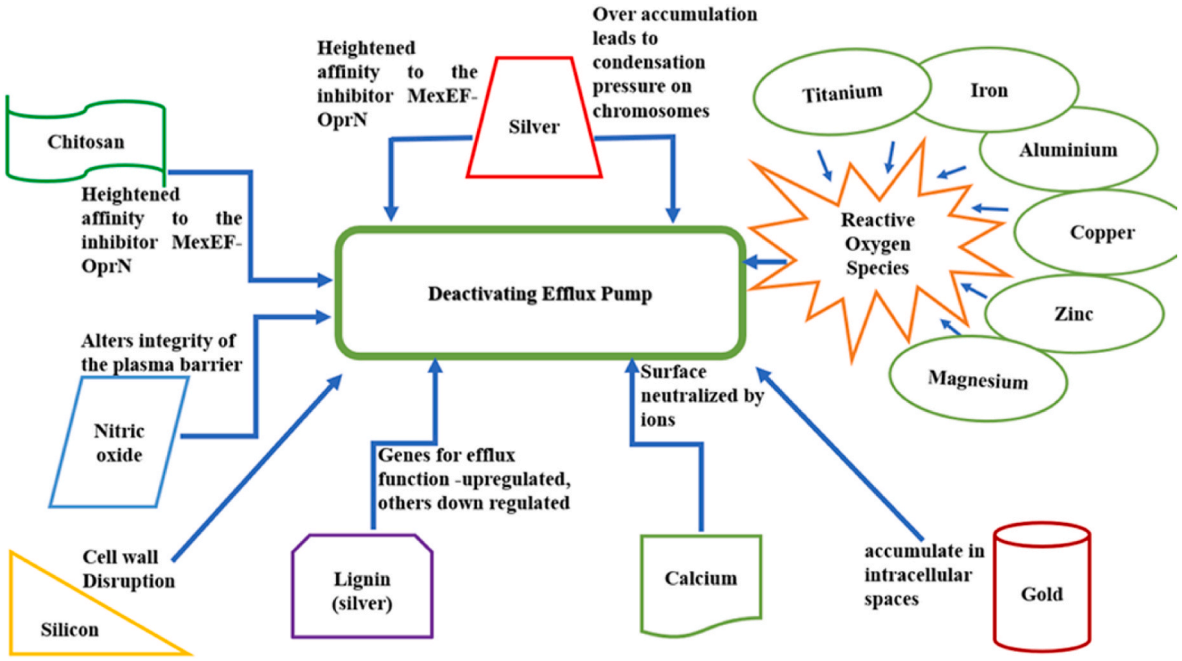

Fig. 1. The graphical representation of the possible nanoparticles that aid in the deactivation of the efflux system.

is done to neutralize the charge variations between the nanoparticles and the host. This host response tries to repel the nanoparticles away from the infectious agent. The host can perform this mechanism successfully if the nanoparticle concentration is kept below the inhibitory levels. If the nanoparticles are administered above the minimum levels, they can create enough charge difference to aggregate the nanoparticles on the surface of the bacteria. This leads to oxidative stress and inevitable damage to the pathogen. When continuous low dosage levels are exposed to a particular pathogen, they act as a stimulus to enhance their survival for the future. Another mode of resistance is attained when the pathogen senses the presence of other species for synergistic growth feasibility. Naturally, *Enterococcus faecalis* is dormant in nature. It is found to be the main cause of infections in dental roots. They have also been seen in several resistant phenotypes (Lemire et al., 2013). When the habitat around the pathogen becomes unfavourable such as deficient in nutrients, they become dormant and cease to grow (Mijnendonckx et al., 2013). When co-species like *Candida* is sensed by *Enterococcus faecalis*, it gets reactivated and develops persistent pathways. The feasible ways for the bacteria to survive and adapt to unfavourable environments consist of a passive mechanism and an active mode of defence. In the former mode, the cell does molecular modifications. These changes can be in the guanosine penta phosphate, tetraphosphate, modules of toxin and its counterpart, indole, etc. while the active mode of gaining resistance is through the efflux systems (Yamanaka et al., 2005). In this current review, the efflux system has been discussed; hence, the active mechanism of gaining persistence is given importance. Pu and team worked on the effect of efflux on the resistant strains and their cellular drug concentrations. They found that the fluorescently tagged antibiotics were found more in the external environment than inside of the cells. These results were very evident in single-cell microscopy. When these special species were sequenced and analysed, their efflux system has shown a group of over-expressive genes. Their rate of pumping the antibiotics out of the cell was also found to be in elevated levels when compared to their normal counterparts (Lee et al., 2010). Nanoparticles in their ionic forms are also pumped out effectively (Nallathamby et al., 2010). The size of the nanoparticles influences the efflux rates based on the species they are employed on.

The charge of the bacteria and the nanoparticles determines the extent of the latter's antibacterial activity. An experimental study was performed on both gram-positive and negative strains having variations in charges. All the three charges of positive, neutral and negative were studied. The gram-positive strains under study were *Staphylococcus aureus*, *Streptococcus mutans* and *Streptococcus pyogenes*. *Proteus vulgaris* as well as *E. coli* were the gram negative bacteria studied. The results s were that the positively charged nanoparticles had the greatest affinity and heightened antibacterial responses when compared to the neutral and negative particles. When size-dependent studies were performed, the outcomes were still influenced by the surface charge than the dimension (Porras Gómez et al., 2012). Along with oxidative stress mentioned in the previous section, osmotic pressure and pH (especially alkaline) also alter the surface charge of the microbe. Sigma factors that work for the extra-cytoplasmic functions lead to oxidative stress. They trigger the expression of many known and unknown genes of multiple functionalities. The most frequent function is to increase the production of lipopolysaccharide layers outside the cell. This affects the charge on the cell and its affinity towards nanoparticles. They also tend to introduce D-alanine amino acid in the membrane matrix to reduce the net negative charge. This step is again helpful for the pathogen to overcome cationic-based medications and therapies. Excessive external stimulus leads to the possibility of mutations. Mutations in the efflux system can elevate the bacterial threshold towards medications and treatments. The reactive oxygen species tend to up-regulate the genes associated with the efflux pumps and reduce the regulation of porins. This makes the bacteria resistant to the specific drug (Tin et al., 2009).

7. Future aspects - species-specific efflux system

To overcome the efflux-based resistance pattern in bacteria, it is essential to focus on the molecular makeup of the pump. Each species has its own set of regulatory genes that the nanoparticles can target to retrieve the susceptibility of the species. A few of the most commonly found infectious pathogens are discussed below for their potential efflux carriers and genes. These genes can be used in the future as targets by the nanoparticles to make the pathogens susceptible to medications.

Gram-Negative – In this group of organisms, nanoparticles can be tailored to focus on RND in the outer membrane of the cell. This efflux system is the primary ejection pump that most of the gram-negative utilize to become persistent in the given environment.

E. coli - The efflux system has AcrAB carriers. Many persistent varieties are due to the mutation of the acrAB gene. They harbour resistance against fluoroquinolone, tigecycline, and cefuroxime. Many carry plasmid mediated efflux genes too. Tet B, C, and K genes have been found in plasmids of *E. coli* that harbor tetracycline resistance. These have been found to be working in flushing away angucyclinone antibiotics. Several species attain Olaquindox resistance because of the OqxAB carrier pump. This whole process is TolC dependent. Various plasmids also have QepA/QepA2 genes to gain resistance against the conventional drugs (Cattoir et al., 2008; Hansen et al., 2004; Källman et al., 2003; Lautenbach et al., 2009).

Salmonella – they contain multiple efflux systems working simultaneously to provide rapid resistance to drugs. Their basic efflux carriers associated would be AcrAB-TolC. Resistance have been successfully attained for ciprofloxacin, cyclohexane, fluoroquinolone, etc. 10% of the population are resistant because of the overexpression of AcrAB. *S. typhimurium* has multiple genes activated for its efflux system. These genes are emr D, B, acrA, B, E, F, mdlB, mdtB, C and A. Mutations can lead to the deletion of TolC and AcrB carriers. When it comes to metal resistance, MdtABC and AcrD are found to be involved. Strains that harboured compromised TolC exhibited a heighted susceptibility. If the AcrAB-TolC carrier is altered, the overall expression of the pathogen is seen to be compromised (Braoudaki and Hilton, 2005; Chen et al., 2007; Nishino et al., 2006; Olliver et al., 2005; Piddock et al., 2000; Randall et al., 2001; Ricci et al., 2006). Some other pumps present in *Salmonella* species for providing MDR are MdfA, EmrAB, MdtK, etc.(Nishino et al., 2007).

Enterobacter – Apart from *E. coli*, the other most common enterobacterium is *Enterobacter aerogenes*. AcrAB-TolC is responsible for MDR in these species. They have harboured resistance against clarithromycin, quinolones, chloramphenicol, erythromycin, tetracyclines, etc. Phenolic active compound resistance was feasible because of the presence of MarA carrier. The resistance to tigecycline is also mediated by the activation of RamA carrier A cryptic form of RND is found in *Enterobacter* species that is encoded by EefABC. These were found to be ineffective to provide persistence against ketolides and fluoroquinolones (Chollet et al., 2004; Masi et al., 2006).

Klebsiella – AcrAB is seen to be overexpressed in its homologous form. Mutations are generally reported in RamA and AcrR that often lead to the overexpression of efflux pumps. They play a pioneer role in the β-lactam based resistance. EefABC has been reported to make *Klebsiella* spp. tolerant to gastrointestinal acids. It is seen that the inactivation of EefA, makes the bacteria susceptible again to several medications and therapy (Coudeyras et al., 2008; Schneiders et al., 2003).

Serratia – *Serratia marcescens* is one among the several naturally occurring resistant strains. They have 3 types of RND pumps SdeXY, SdeAB-HasF and SdeCDE. The analogue to TolC is HasF carrier. In plasmids, three types of genes encode the efflux pumps. These are SsmE, SmdAB and SmfY. In the natural habitat, TetA (41) is seen in *S. marcescens* (Begic and Worobec, 2007; Matsuo et al., 2008; Stock et al., 2003).

Vibrio – they have 6 types of operons for the activation of efflux pumps. They have an RND-type pump. Among the 6, 2 are bile regulated, namely, VexCD and VexAB. These provide the bacterial resistance to bile. Hence, the bacteria survive well in the gut. VexEF confers to provide persistence against antibiotics. Many strains of vibrio have shown the presence of MATE pumps. Very few harbours ABC pumps. Efflux system is the major reason that *Vibrio fluvialis* is highly resistant to quinolone (Bina et al., 2006) ((Huda et al., 2003; Srinivasan et al., 2006).

Pseudomonas – these mostly contain soil bacteria. They have the tripartite system that works as an efflux system. Mex pumps are responsible for the survival of *Pseudomonas aeruginosa*. They are a type of RND pump. MexXY-OprM and MexAB-OprM are found to be overexpressed in several resistant varieties of *Pseudomonas* that cause blood infections (Driscoll et al., 2007; Strateva et al., 2007; Sugawara et al., 2006). Novel systems have been reported recently for the same strain. They are TriABC-OpmH, MexMN-OprM, MexVW-OprM and MexPQOpmE. MATE type pumps are also seen, which are denoted as PmpM (Zhou et al., 2006).

Acinetobacter - Among the different strains available *Acinetobacter baumannii* has emerged as the most widely spread MDR strain in the world. RND has been reported for these species. AdeIJK is found to be the carrier involved in the process. Some additional genes reported are AdeXYZ and AdeDE. MATE, MFS and SMR are also documented for the same species (Bergogne-Berezin and Towner, 1996; Chu et al., 2006; Gilad and Carmeli, 2008; Su et al., 2005).

Gram Positive-these are the population of bacteria that are known to have peptidoglycan layers in their cell membranes. They seem to have lower resistance patterns than the gram-negative counterparts. A few well-known strains and their efflux mechanisms that can be addressed in future by nanotechnology and therapy, are discussed below.

Clostridium – these are gram positive strains that are found in various infections. They are anaerobic in nature. There are 4 main pathogen varieties of *Clostridium* that have shown an efflux pattern of resistance. These are *Clostridium difficile*, *Clostridium tetani*, *Clostridium botulinum* and *Clostridium perfringens*. Among the 4, *Clostridium difficile* has been shown to have the maximum hospital acquired resistance pattern. Most of the higher generation antibiotics used in hospitals tend to make these pathogenic strains resistant. It has been reported that cdeA is responsible for the MATE type efflux pump in *Clostridium difficile*. These pumps are sodium ion coupled pumps. If this gene is found to be expressing in the plasmid DNA, then the resistance pattern is successfully conjugated to *Clostridium perfringens*. Cme is also found to be a gene responsible for efflux in *Clostridium difficile*. They are homologues of MefA/MefE. These are able to conjugate their resistant patterns to *Enterococcus faecalis* too. A mutation in the gyrB had s also reported tremendous resistance patterns that were not affected by the presence of efflux inhibitors. Tet (40) and Tet(P) are also found to be the part of the efflux expressing genes in *Clostridium* species (Kazimierczak et al., 2008; Owens et al., 2008).

Bacillus- It is found to have multiple pumps that aid the pathogen to remove the toxic materials as well as antibiotics out of the system. A few common ones are Bmr3, Bmr and Blt. It has a fourth drug efflux system that gets expressed due to the mutations of the LmrB gene. Dimer shaped efflux is also found to be present in several *Bacillus subtilis* species. This is represented by Tet(L), which is a tetracycline efflux system. An ABC transporter has been discovered in *Bacillus subtilis* species that is functional in nature and is denoted as YvcC (BmrA) (Bast et al., 2004; Ohki and Tateno, 2004).

Listeria monocytogenes – they are pathogens found in contaminated foods. They have been susceptible to many medications for years. Nevertheless, recently, these strains have started harbouring some form of resistance. They have tet(K), floR and tet(A) as efflux carriers on their membranes. MdrL is found to be a partial transporter that can cause resistance patterns in these strains (Ramaswamy et al., 2007; Soumet et al., 2005).

Staphylococcus – Efflux is a very important technique opted by *Staphylococcus aureus* to thrive against different medications. It has separate genes in the chromosomes and the plasmid to express and activate their efflux systems. In the chromosome, NorA genes are found. In the plasmid, QacA/B and MsrA are found. Few more additional genes encoding for pumps are Tet (38), MdeA, NorB, MepA, AbcA. SepA, NorC and SdrM. They have almost all types of efflux carriers available to help the organism with the ejection of drugs from its internal compartments. In cases of abscess, NorB is seen to overexpress the pathogen to make it highly tolerable to medications (Ding et al., 2008; Hassan et al., 2007; Truong-Bolduc and Hooper, 2007).

Enterococcus faecalis - *Enterococcus faecalis* is another gram-positive strain that is very much resistant to several antibiotic drugs. Its r mode of efflux-based resistance is an ABC transporter. EfrAB is the gene associated with it. Lsa genes are also associated with potential resistance

patterns in *Enterococcus faecalis*. Few drugs such as norfloxacin tend to instil mutations in bacteria that have non-EmeA strain, which is similar to NorA pump. Majority of them will contain msrC. This gene encodes for the elimination of erythromycin (Aslangul et al., 2006; Lee et al., 2003).

Streptococcus pneumoniae – this pathogen is very well known to cause various *pneumococcal* related illnesses. Efflux is exhibited in most of the *Streptococcus* species. Its efflux is generally of MFS type. These are denoted as PmrA, MefA and MefE. PatA and B are also suggested to be a part of the ciprofloxacin resistance in *Streptococcus* species. These are a type of ABC transporter proteins. A similar type of gene i. e SP2073 to 75 is also noticed in the strain that regulates the ejection of some quinolones. . If the inactivation of other carriers for efflux such as MATE and MFS is initiated, *Streptococcus* doesn't show much change in the susceptibility trend. Mef(A) gene is also reported to be the causative agent for the presence of efflux mechanism in M phenotype *Streptococcus* species (Cousin et al., 2003; Jumbe et al., 2006; Marrer et al., 2006). Table 3 depicts the nanoparticles used till date to treat potential infectious pathogens that use efflux systems for their survival.

Size seems to play a major role when it comes to efflux hampering by metallic nanoparticles. Smaller particles reside longer within the pathogenic cell when compared to their larger counterparts. Hence manifesting size influenced efflux kinetics of the membrane pumps (Banerjee et al., 2010). In *P. aeruginosa*, it was reported that a single nanoparticle tends to have a higher accumulation in the internal components of a wild-type strain when compared to MexAB-OprM overexpressed strains. While further investigating, it was found that the residual time of the nanoparticles increase (up to 10 times) when given in combination with an efflux inhibitor like Carbonyl Cyanide m-Chlorophenylhydrazine (CCCP) or any form of medication (Nallathamby et al., 2010). When the drug concentration is administered in optimal amounts in conjugation with nanoparticles, the efficacy has been quite remarkable. The efflux pumps extrude aztreonam when administered in lower quantities (0–3.13 μg/mL). At optimal concentrations (31.3 μg/mL), the drug alters the cell membrane to the extent that the accumulation of the nanoparticle becomes 8-fold, hence killing the pathogen. A similar effect has been seen between chloramphenicol, amoxicillin, clindamycin, erythromycin, penicillin G, vancomycin, and silver nanoparticles (Nallathamby et al., 2010; Okkeh et al., 2021). Thus, its not only the nanoparticle that aids the drug but vice versa is also seen in many strains.

The overall bactericidal activity of the antibiotic is enhanced when used in combination with nanoparticles. In 2007, Saha and team streptomycin, kanamycin or ampicillin conjugated gold nanoparticles remarkably reduced the minimum inhibitory concentration against a broad spectrum of pathogens (Saha et al., 2007). Similarly, in 2017, gold nanoparticles with fluoroquinolones worked very efficiently against MDR *E. coli* (Gupta et al., 2017). The unique structure of the nanoparticles gives an advantage over commercial drugs in terms of antibiotic resistance (Zazo et al., 2016). When tailored and packed with various medications, resistance is unlikely to occur in a pathogen as spontaneous and consecutive mutations would be required by the microbe to survive (Zhao and Jiang, 2013). The efficiency of silver nanoparticles with antibiotics against beta-lactamase *E. coli, Staphylococcus aureus, Pseudomonas aeruginosa*, etc., is quite effective in lower concentrations (Scandorieiro et al., 2016). The combination of antibiotics with nanoparticles seems to reverse the resistance in a pathogen and increases the efficiency of many drugs like clindamycin, ceftazidime, erythromycin, ciprofloxacin, vancomycin, polymyxin B, ampicillin, methicillin, etc. (Hemeg, 2017). But these studies have been reported mostly in lab isolates or animal models. Translations of the same in human clinical trials have not yielded much clinically significant changes in the overall metabolic, urine and haematological profile of the test subject. Healthy volunteers were administered with 10 ppm and 32 ppm of silver nanoparticles ranging from sizes 10 nm to 40 nm respectively. This brings into attention the need for optimization of nanoparticle dosage in humans (Munger et al., 2014).

8. Limitations and future perspectives

Limitations involving the utilization of nanomaterials as a potential remedy to tackle efflux pump-based resistance are that these particles themselves harbor unpredictable hazardous traits to the biota around them. Continuous exposure of the microbial population to high concentrations of nanomaterials can lead to the attainment of another undesired trait by the pathogen, which needs to be addressed later. The best perspective that could be followed while working with nanomaterials for efflux impediments would be tailoring the nanoparticles very specific to the pump and protein associated with it. This would lead to very lower dosages of nanoparticles yielding remarkable outcomes in making these potential pathogens susceptible to lower generations of antibiotics again. The genes expressing the pumps can be specifically targeted by the designed nanoparticles to solve the multiple drug resistance issue at a very primary level. The therapeutic ability of nanomaterials is quite limited to its clearance, mode of action, nature of the target, dosage, physicochemical, morphological, chemical properties, the solvent used, environmental factors, and toxicity generated against the pathogens (Beyth et al., 2015). Very specific and optimal combinations of nanomaterials with parent antibiotics can avoid the emergence of drug resistance. But the translation of this experimental hypothesis into clinical evidence requires detailed perception with proper implementation of pharmacodynamics and pharmacokinetic studies. It has been reported previously that there are a synergistic effect between antibiotics conjugated nanomaterials, especially metallic nanoparticles (Baptista et al., 2018; Pelgrift and Friedman, 2013). Among the various features opted by a nanoparticle against microorganisms, controlling the efflux system is one of them. The large aspect ratio of the materials increases their contact with the pathogen hence deactivate the efflux system in many ways (Durán et al., 2016; Hemeg, 2017).

Currently nanoparticles are effectively used as surface coatings and dermal therapeutics. Scale-up and production guidelines for manufacturing nanoparticle and antibiotic conjugations need to be laid with efficient standardization of toxicology assays and protocols. Efforts are to be taken for optimizing the drug dosage for oral administration in humans so that the drug nanoparticle combination can reach the pharmaceutical market and cease the rise of MDR (Zaidi et al., 2017). Lastly, the economic impact of the clinical translation of this combination must be addressed in terms of efficiency in humans (Zazo et al., 2016).

9. Conclusion

The current review article focuses on the effects of nanoparticles on efflux systems. Many nanoparticles have shown promising results in labs and bench-scale studies. Their outcomes have been so remarkable that several patents are also filed to scale them up for commercial applications. However, when it comes to the clinical implementation of these nanoparticles, there are numerous challenges that need to be tackled. Even if the clinical translation of the particles becomes a success, the risk assessment, especially, time-based assessment is always an unanswered question. Assessment of viable cells and systems varies from the models used. It is always essential to know about the various modes these particles opt to imply their antibacterial activity. Their physical dimensions offer many distinctive advantages to various ineffective drugs. They have been successfully conjugated with medications used in all areas of the body from dermal to gastrointestinal. The main efflux carrier found to have a primary responsibility to provide resistance is the RND carrier system. Secondly, MATE and ABC carriers with mutations also result in persistent bacterial species. The major mechanisms of their activities have been discussed in the present article. They mainly focused on the disruption of the cell wall or damaging the subunits of the carrier system. Very little evidence is there for the manipulation of genetic makeup

Table 3
Different Nanoparticles used to treat Efflux based potential infectious pathogens.

Category	Nanoparticle	Organism	Mean Shape	Average Size/ Concentration range	Average MIC value	Mechanism	References
Polymer	Chitosan	*E. coli* and *P. aeruginosa*	N/A	Below 500 nm	–	Heightened affinity to the inhibitor MexEF-OprN overexpression inhibited	(Helander et al., 2001; Lee et al., 2019; Pelgrift and Friedman, 2013; Tin et al., 2009; Usman et al., 2013; Yang et al., 2021)
	Lignin with silver	*S. aureus, S. epidermidis, P. aeruginosa, K. pneumoniae,* and *A. baumannii*	shells	42 nm	5–25 μg/mL	Genes encoding for membrane proteins with an efflux function were upregulated, others down regulated	Slavin et al. (2021)
Non metal	Nitric oxide	*K. pneumoniae, S. aureus*, *E. faecalis*, *E. coli, P. aeruginosa*	N/A	1.25–5 mM	–	Altered integrity of the plasma barrier	(HuhandY and Kwon, 2011; Lee et al., 2019; Raafat et al., 2008)
Low-cost metal	Zinc	*A. baumannii*	Rod, spheres	10–40 nm	20 μg/mL	breakdown of the membrane integrity and generation of reactive oxygen species efflux system (NorA) falls short of energy	(Banoee et al., 2010; Couto et al., 2008; Ghasemi and Jalal, 2016; Lee et al., 2019; Venubabu Thati et al., 2010; Yang et al., 2021)
	Magnesium	*S. aureus* or *B. subtilis*	N/A	Average 100 nm	–	generate reactive species of oxygen	(Hajipour et al., 2013; Lee et al., 2019; Pelgrift and Friedman, 2013)
	Copper	*S. aureus*, *E. faecalis* and *E. coli*	Spherical, quasi spherical, rod, rectangle	Less than 300 nm	E. coli- 20 μg/ mL S. aureus was 140 μg/mL	stop production of biofilms and enhance sensitivity	(Bagchi et al., 2013; Christena et al., 2015; Ruparelia et al., 2008; Yang et al., 2021)
	Iron	*M. smegmatis*	N/A	1–100 nm	–	oxidation of the intracellular components	(Lee et al., 2008, 2019; Sies, 1997; Xing et al., 2013)
	Aluminium	*E. coli*	N/A	10–100 nm	–	Cell wall disruption	(Lee et al., 2019; Zaidi et al., 2017)
	Titanium	*S. aureus*, *E. faecalis* and *E. coli*	N/A	30–45 nm	–	oxidation of the intracellular components	(Hemeg, 2017; Lee et al., 2019; Rudramurthy et al., 2016)
Mineral based Nanoparticles	Calcium	*S. mutans* and *E. faecalis*	spheres	–	–	The binding of the ions to the anionic moieties of the cell surface, neutralize them and decreases the repulsion forces between them	(Drummond, 2008; Lee et al., 2019; Lim et al., 2002)
Quartz Nanoparticle	Silica	*S. aureus*	crystals	Less than 400 nm	–	Cell wall disruption	(Lee et al., 2019; Zaidi et al., 2017)
Noble metals	Platinum	*P. stutzeri, P. aeruginosa, Lactobacillus* sp.	Spheres	1–3 nm 4–21 nm	5 μg/mL	Exhibited good antibacterial activity, mode for efflux deactivation yet to be investigated	(Gopal et al., 2013; Lee et al., 2019; Rezaei-Zarchi et al., 2012; Yang et al., 2021; Yoshihisa et al., 2010)
	Silver	*P. aeruginosa, B. subtilis, S. aureus, S. typhi luteus, Micrococcus* sp,	Spherical, rod, polyhedron	Less than 100 nm	33 nM	MexAB-OprM overexpression inhibited Over accumulation of the nanoparticle leads to condensation pressure on the chromosomes	(Bera et al., 2014; Kim et al., 2007; Kyriacou et al., 2004; Lee et al., 2019; Li et al., 2005; Xu et al., 2004; Yang et al., 2021)
	Gold	*Enterococcus* sp. *E. coli* resistant to vancomycin, *Pseudomonas* species resistant to methicillin *Corynebacterium*	Spheres, nanorod	Less than 100 nm	5 μg/mL	accumulate in the intracellular space damage to the efflux pump	(Bahrami et al., 2014; Gu et al., 2003; Hayden et al., 2012; Hyland et al., 2006; Khan et al., 2016; Lee et al., 2019; Mohamed et al., 2017; Okkeh et al., 2021; Rosi et al., 2006; Yang et al., 2021; Zhao et al., 2010)

in the efflux system. The present review has provided the compiled data about the possible genes targeted using nanoparticles to aid the current antibiotics to function against many resistant species. Novel efforts are underway to incorporate these particles in medical devices and equipment to avoid microbial growth and proliferation. However, systemic administration of nanoparticles is still not accepted to a great extent. Proper guidelines are yet to be devised logically and rationally to optimize and form these nanoparticles against MDR species. These norms should cover the whole aspect and the process involved in the making of drug nanoparticles. The modes of characterization, the properties to be considered, the steps and features for biocompatibility and norms for data comparison and acquisition in both lab and clinical studies should be addressed well in the future. Suppose nanoparticles are made target specific to the carriers of efflux and genes for the respective species. In that case, the conventional medications will have better efficacy in treating patients worldwide. The load on generating new and novel drugs will reduce, and the cost of treatment will come down as older medications would be put to use again. The study on the targeting of the nanoparticles towards these specific genes and efflux carriers should be of utmost preference in medical microbiology in the upcoming years.

Author statement

Nibedita Dey: Conceptualization, Methodology, Writing – original draft. Kamatchi C: Writing – original draft. Vickram A S: Writing-Reviewing and Editing. Anbarasu K: Writing-Reviewing and Editing, Thanigaivel S: Writing-Reviewing and Editing, Jeyanthi Palanivelu: Writing-Reviewing and Editing, Arivalagan Pugazhendhi: Writing-Reviewing and Editing, Vinoth Kumar Ponnusamy: Supervision, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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