Metal nanoparticles and consequences on multi-drug resistant bacteria: reviving their role

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Abstract

The most paramount challenge faced globally by the physicians is to treat the infections caused by nosocomial multi-drug resistant microorganisms. These bacteria are endowed with unique efflux pumps, which are substrate specific and a major defense mechanism for the bacteria. In fact, among various etiologies, the expulsion of antibiotics through efflux pump/s stands alone as an important factor for such resistance which these bacteria develop with time. To unravel this problem, several efflux pump inhibitors (EPI), which augment the efficacy of antibiotics, have been proposed to be administered along with antimicrobial agents nevertheless, none of them have entered in clinical use yet. In this review, we provide an overview of some of the nanoparticles postulated against multidrug resistance efflux pumps, and the inhibition strategies of EPIs action of some of the nanoparticles was searched along with EPIs which have been postulated for blocking the efflux pumps.

Keywords Nanoparticles · Efflux pump inhibitors · Multi-drug resistance · Efflux pumps

1 Introduction

Efflux pumps play a significant role of drug detoxification in various type of bacteria [1]. Evolution of efflux pumps is still in the debate, with few researchers believe that these pumps emerged due to the bacterial stress while others retain faith in their ancestral origin [2, 3]. Nevertheless, the theme preserves its uniqueness in that they are present in antibiotic susceptible as well as resistant bacteria and may be specific or act on several substrates [4]. Their enhanced expression may even give rise to multi-drug resistance. Resistance towards several antibiotics is a

medical apprehension particularly when the field of vision is nosocomial infections [4]. Efflux pumps are grouped in five families comprising: major facilitator superfamily (MFS), ATP-binding cassette (ABC), small multidrug resistance (SMR), resistance-nodulation-division (RND), and the multidrug and toxic compound extrusion (MATE). Depending on their group families they are single-component transporters or multiple-component systems [5]. The single-component transporters containing inner membrane transporter such as MATE and SMR type efflux pumps and the multiple-component systems containing inner and outer membrane channel and a membrane fusion

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protein, such as the RND type efflux pumps that couple to proton motive force. Their tripartite arrangement allows the bacteria to direct extrusion of toxic drugs from cytosol or periplasmic space to the outside of bacterial by using the proton-gradient as an energy source [2, 6]. The expression of RND pumps is often regulated by local regulators (encoded upstream of the operon) and global regulators (like bile salt and fatty acid) [6].

RND and MFS families of efflux proteins have an imperative role in the intrinsic drug resistance among gram-negative and gram-positive bacteria [5–8]. RND, ABC and MFS family efflux pumps can transport drugs by a three-step rotating mechanism in gram negative bacteria. Structural and functional of efflux pumps was shown in Fig. 1. At first potential substrates have access to enter when the entrance is kept open and the substrates move through the channel made by membrane fusion protein [9]. Then, in the efflux state, the entrance is locked and the exit is unlocked because of the conformational changes of the efflux pump that coupled to catalysis in F_1F_o -ATPase or the proton motive force [2, 9]. After the extrusion of the substrate, the efflux pump turns back to the Access state for the other substrate [9]. RND system can efflux aminoglycosides, β -lactams, chloramphenicol, erythromycin, and tetracycline and ethidium bromide and reduce susceptibility to fluoroquinolones [10]. MFS system can efflux tetracycline, minocycline and chloramphenicol. MATE family can extract norfloxacin, ofloxacin, ciprofloxacin, gentamicin, daunorubicin, doxorubicin, rhodamine 6G and ethidium bromide [10]. RND system is a part of the bacterial protection system against both reactive oxygen species (ROS)

and the host defense mechanisms. Adjunct to this, they play an effective role in the virulence of bacterial pathogens including; Colonization, twitching motility, toxin and biofilm production [6, 11]. Hence, the alternative for the future is the identification of molecules that inhibit efflux pumps so as to reduce drug resistance and bacterial pathogenesis [6, 11].

Efflux pump inhibitor (EPI) are described as weapons of anti-resistance by acting as an adjuvant of antibiotics, inducing an effect on the efflux pumps but retaining the activity of an antibiotic [5, 7]. Many compounds have been tested and suggested for their efflux pump inhibition ability including some analogues for antibiotic substrates and other chemical compounds, but few of them take into consideration the structure–activity relationship and the spectrum of the activity [12]. They block the efflux family pumps as combinational therapy [13]. Efflux pump inhibitors (EPI), which may be natural or chemically synthesized compounds are usually considered only for their properties as antibiotic adjuvants, while their anti-virulence potential is seldom taken into account [12, 14]. Interestingly, EPIs are also used as an indicator to confirm the presence of active efflux pumps, for example in *Pseudomonas aeruginosa* [15].

EPI activity of phenylalanine arginyl *b*-naphthylamide (PA β N) is evidenced to counteract over expression of MexAB, MexCD, and MexEF efflux pumps in *P. aeruginosa* and other efflux pumps in gram-negative bacteria including *Escherichia coli*, *Enterobacter aerogenes*, *Klebsiella pneumoniae* and *Salmonella enterica* [13, 16–19]. Phenothiazines and their derivatives such as chlorpromazine

Fig. 1 Residing time of AgNPs inside *P. aeruginosa* over expressed MexAB-OprM alone and in combination with different concentration of chloramphenicol and aztreonam. It is determined that overcoming to efflux pump by silver nanoparticles is antibiotic dose-dependent manner

or thioridazine are other classes of EPIs substantiated to reduce antibiotic resistance in *Burkholderia pseudomallei* and *S. enterica* [20, 21]. Amongst EPIs, heterocyclic derivative of benzochromene (BC9) has been identified as a specific NorA efflux pump (MFS family) inhibitor thereby mitigating ciprofloxacin resistance. Concomitant use of antibacterial agents with EPIs has been suggested to reinvigorate antimicrobial agent as well as provide a synergistic effect and assist to combat with over-expression of efflux pumps in the resistant bacteria. However, none of efflux pump inhibitors have entered a clinical usage yet [16, 19]. Verapamil, an efflux inhibitor, has been profoundly found to decrease the MICs of bedaquiline and clofazimine in *M. tuberculosis* by 8- to 16-fold. Since the efflux pump mediated resistance is a significant antibiotic resilient mechanism in these bacteria, verapamil can be opted as an adjunctive chemotherapeutic agent for *M. tuberculosis* [22]. Another strategy revised for decreasing efflux pump activity is the disruption of the bacterial proton-motive force by using 2,4-dinitrophenol (DNP) and carbonyl cyanide m-chlorophenylhydrazone (CCCP) [23]. Similar to other EPIs, quinazoline has been found to possess an excellent activity to reduce the MIC of nalidixic acid, ciprofloxacin and chloramphenicol in gram-negative bacteria and to decrease overexpressed efflux pump AcrB, MexB and NorA activity in MDR strains [13, 19, 24]. The blocking activities of arylpiperazines compounds such as NMP on RND-type efflux pumps are modulated by the spatial structure of these compounds [13, 25]. NorA-(as a MFS family) and MepA-efflux pumps (as a MATE family) are shown to be inhibited by paroxetine which is one of the phenylpiperidine selective serotonin reuptake inhibitors (SSRI) [26]. Liposome is another promising EPI agent. Fusion of liposome and an antibiotic execute the release of antibiotic into the bacterial cell forthwith [27].

Ethanol as a crude plant extract from *Mentha avensis* and other extracts from *Turnera ulmifolia* are some of the examples to take over the antibiotic resistance in clinical isolates of *E. coli* [28, 29]. Lemongrass oil (*Cymbopogon citratus*) has been shown to possess synergistic effect when used in conjunction with kanamycin and streptomycin on *Salmonella typhimurium*. Lemongrass oil and 50-methoxy-hydnocarpin produced by barberry plants has been shown to block NorA pump in *Staphylococcus aureus* [13, 30]. However, the main element from these extracts which inhibit efflux pump has not yet been identified [13].

Richard Feynman first theorized nanotechnology and is renowned as a Nobel-prize winner in 1959 [31]. Aftermath of his achievements made science of nanotechnology to creep as a new strategy to overcome the snags of antibiotic resistance [32]. For enhancing the therapeutic effectiveness of antibiotics, these have been encapsulated with nanoparticles [33]. Nanoparticles within the range size

between 1 and 100 nm and high surface-to-volume ratio have an antimicrobial effect on MDR pathogens, which has been described as change in the membrane permeability, inhibition of multidrug efflux pumps, and damage to the chromosome [33, 34]. They combat with bacteria by using multiple mechanisms, thus emergence of resistance to them is probably unlikely [34, 35]. The main antimicrobial effects of metal nanoparticles are due to the release of ions and production of reactive oxygen species (ROS) [34] such as superoxide ($O_2^{\cdot-}$) ascending from electron transport processes of bacteria that is modified to hydrogen peroxide (H_2O_2) by superoxide dismutases (SOD) [36]. H_2O_2 is reduced to reactive hydroxyl radicals by redox-active metals (Fenton chemistry) [37–39]. They can damage DNA, RNA, cell membrane, proteins and bacterial oxidative phosphorylation and finally causing bacterial death [40]. The released ions can cause structural damage by creating hole in the bacterial membrane and thereby decrease the membrane integrity. They also interact with H^+ gradient of the membrane. So, all energy dependent mechanisms in bacterial membrane such as the ABC super family efflux pumps and the others are suppressed followed by lethality of the bacteria [34].

2 Silver

Silver gained antibacterial feature since the ancient Greeks and its nano-crystalline form has demonstrated an unsurpassed antibacterial spectrum with efficacy on 150 different bacterial pathogens [41–43]. For centuries silver based compounds have been applied in the treatment of burn and chronic wounds and also used as eye drops for the prevention of trachoma [43]. It is also used as a silver-impregnated polymer in catheters for preventing the bacterial biofilm growth and in topical creams for healing burn wounds [41–43]. The antimicrobial effect of silver compounds is well documented, however, the inhibitory mechanism of silver on microbial growth is only partially known. It is suggested that the antimicrobial effect of silver nanoparticles is a combination of several reactions; penetration of the cell membrane causing structural changes in its permeability that cause accumulate of nanoparticles into the cell. So generation of free radicals is happened by ROS activation leading to a porous cell membrane, deactivation of vital enzymes, destruction of microbial DNA and RNA and finally the cell death of pathogenic microorganisms [44]. Silver ions interacts with chemical group of polymers present in bacterial membrane, reducing their integrity [45–47]. These ions can block electron transfer mechanism of bacteria by interacting with thiol groups of cysteine residues of NADH: quinone oxidoreductase (NQR) and

inhibit its activity, failing many energy dependent mechanisms in bacteria [41, 48]. Silver ions have also been demonstrated to interact with nucleic acid and prevent its replication or even promote production of reactive oxygen species (ROS) that damages proteins, DNA, RNA and lipids [41]. For as much as silver nanoparticles (AgNPs) can produce further ionic silver, their ability for damaging bacterial cell wall, cell membrane proteins, DNA, RNA and lipids are increased [49–51]. Xu et al. [52] and Lee et al. [53] independently synthesized and characterized purified spherically shaped silver nanoparticles (Ag NPs), and used the size-dependent localized surface plasmon resonance (LSPR) spectra of single Ag NPs to determine their sizes and to probe the size-dependent transport kinetics of the ABC transporters in single living cells (*Bacillus subtilis*) and *P.aeruginosa* respectively in real time at nanometer resolution using dark-field optical microscopy and spectroscopy (DFOMS). They showed the smaller NPs reside longer inside the cells than larger NPs, suggesting size-dependent efflux kinetics of the membrane transporter [52, 53]. Similar research on size-dependent plasmonic spectra of single NPs to probe the size-dependent transport kinetics of MexAB-OprM (multidrug transporter) in *P.aeruginosa* in real-time at nanometer resolution later confirmed that the accumulation of intracellular NPs in wild-type (WT) cells was higher than in over-expressed MexAB-OprM, but less than Δ ABM (deletion of MexAB-OprM) [54]. They further demonstrated that residual time of NPs inside the cells increased in the presence of efflux pump inhibitor, Carbonyl Cyanide m-Chlorophenylhydrazine (CCCP) [54]. Other investigators studied the effects of AgNPs in the presence of chloramphenicol and aztreonam in *P. aeruginosa* which comprised several types of expression of MexAB-OprM [49, 52, 55]. Overall, these researchers investigated the role of the MexA-MexB-OprM efflux pump in controlling of the function of aztreonam (AZT) and chloramphenicol, in two mutants, nalB-1 (a mutant that overexpresses MexAMexB-OprM) and Δ ABM (a mutant devoid of MexAMexB-OprM). In the absence of AZT, in wild type (WT) strain, very few nanoparticles accumulated in nalB-1 and Δ ABM. The number of the nanoparticles remains almost unchanged over time. In 3.13 μ g/mL AZT, unlike WT, very few Ag nanoparticles were observed in nalB1, and the number of Ag nanoparticles in nalB-1 remains unchanged over time. In contrast, the number of Ag nanoparticles accumulated in Δ ABM increases proportionally with time. In 31.3 μ g/mL AZT, the number of Ag nanoparticles in nalB-1 and Δ ABM increases with time rapidly as observed in WT. Taken together, the results suggest that MexA-MexB-OprM plays an important role in the accumulation of the nanoparticles in the cells. Observations in these three strains,

suggest that MexAMexB-OprM appears to effectively extrude nanoparticles at low AZT concentrations (0–3.13 μ g/mL), at which concentrations the cellular membrane is still intact and its permeability is low while, at higher AZT concentration (31.3 μ g/mL), the cellular wall is destroyed. Thus, the extrusion pump is unable to overcome an overflow of substrates (AZT and nanoparticles), and the number of Ag nanoparticles accumulated in any of these three strains increases rapidly with time, at a rate of 6–8-fold higher than in the absence of AZT. Similar results were demonstrated when the number of Ag nanoparticles accumulated in all three strains increased with the chloramphenicol concentration and incubation time, suggesting that chloramphenicol increases membrane porosity and permeability. In addition, the results indicate that accumulation kinetics of intracellular nanoparticles is associated with the expression levels of MexAB-OprM. The mutant with the overexpression level of MexAB-OprM (nalB-1) accumulates the least number of intracellular Ag nanoparticles, whereas the mutant devoid of MexAB-OprM (Δ ABM) accumulates the greatest number of Ag nanoparticles. This suggests that MexAB-OprM plays a critical role in controlling of accumulation of intracellular Ag nanoparticles. These results are shown at Fig. 1. In the absence of the antibiotics, a lot of small AgNPs were observed in the WT (wild-type bacteria) and Δ ABM (deletion of MexAB-OprM), but few of intra cellular AgNPs were found in nalB-1 (over expression of MexAB-OprM) [52]. The staying time of AgNPs were increased on 10 fold concentration of 25 μ g/mL chloramphenicol in the WT and Δ ABM strains, however, they were not changed in the nalB strains. The highest staying times of AgNPs were found in 250 μ g/mL concentration of chloramphenicol in nalB strains. These results suggest that the permeability of the cellular membrane can be raised by increasing the chloramphenicol concentration [52]. AgNPs were found very few in nalB-1 and Δ ABM, unlike WT, in the presence of 3.13 μ g/mL aztreonam. In the presence of 31.3 μ g/mL AZT, the number of AgNPs in nalB-1 and Δ ABM increased with time like as observed in WT [55]. These results suggest the efflux pumps do not work properly because the cellular wall is damaged in the high concentrations of AZT that bind with nano particles. [55]. Figure 1 displays these results distinctly. Overall, Nancy and her colleagues showed that MexAB-OprM plays an important function in the gathering of NPs inside or efflux out of cells. These effects of efflux pumps can be reduced when NPs and antibiotics are used simultaneously [52, 54, 55]. Li with his colleagues in year 2005 studied synergistic antibacterial effects of β -lactam antibiotic combined with silver nanoparticles and found that nanosilver surrounded by antimicrobial groups can cause more

destructive permeability and may act on the respiration functions of bacterial cell membranes [56]. Parallel to this, other investigations demonstrated that silver nanoparticles in combination with penicillin G, amoxicillin, erythromycin, clindamycin and vancomycin had a significant synergistic effect on the growth inhibition of bacteria with a considerable decrease in requirement dosages and their toxicity [56, 57]. Similar studies were followed by other researchers who loaded silver nanoparticles with antibiotics and used them against *S. aureus*, *Micrococcus* spp., *E. coli*, *Salmonella typhi* luteus and *P. aeruginosa* and could show significant growth inhibition [50, 58]. Proteomic and TEM analysis demonstrated that AgNPs cause bacterial death because of membrane damages and collapse of the proton motive force [53, 59]. The antibacterial activity and mechanism of silver nanoparticles (Ag-NPs) on *Staphylococcus aureus* was studied in China and the results showed an increase in the concentration of silver nano-particles with time makes cell DNA to condense to a tension state, loss of their replicating abilities, leading to the release of the cellular contents into the surrounding environments. Further, the investigation found Ag-NPs could reduce the enzymatic activity of respiratory chain dehydrogenase and with the proteomic analysis showed that the expression of some proteins was changed in the treated bacterial cell with Ag-NPs, namely formate acetyltransferase, aerobic glycerol-3-phosphate dehydrogenase, ABC transporter ATP-binding protein and recombinase A protein [60]. Effect of copper (Cu) and Ag nanoparticles has been shown to impair respiration of *E. coli* cell membrane leading to the damage in the bacterial membrane

and decrease in the efflux pump activity [61, 62]. Figure 2 shows antimicrobial activities of silver nanoparticles evidently.

3 Zinc oxide

Zinc oxide nanoparticles (ZnO-NPs) harbors the potentiality to disrupt the cell membrane, generate the reactive oxygen species (ROS) and interact with DNA, RNA, proteins and lipids [63]. In a search for the agents to inhibit or take over the antibiotic resistance, ZnO nanoparticles were found to have synergistic effects with ampicillin, gentamicin, oxacillin, cloxacillin, amoxicillin, cephalexin, cefotaxime, ceftazidime, vancomycin, streptomycin, erythromycin, clindamycin, erythromycin, clindamycin, and tetracycline [64] whereas, in an another investigation these nanoparticles were found to lower the antibacterial activity of amoxicillin, penicillin G, and nitrofurantoin in *S. aureus* while, enhancing the antibacterial activity of ciprofloxacin in the presence of ZnO nanoparticles in both *S. aureus* and *E. coli* test strains have been seen. They proposed ZnO nanoparticles interfere with electron transfer kinetics that is energy source of NorA efflux pump. ZnO nanoparticles blocks this efflux pump to overcome the resistance towards fluoroquinolones in *S. aureus* [21, 65]. They also suggested that ZnO nanoparticles can interacts with membrane Omp protein. Omp protein is responsible for the constraint in the penetration of quinolones to the cell membrane so that ciprofloxacin absorption increases in the cell [65]. Furthermore, it is suggested that the inhibition of the efflux pumps and disruption of the

Fig. 2 Structural and functional of five families of efflux pumps was shown. Major facilitator superfamily (MFS), small multidrug resistance (SMR) and resistance-nodulation-division (RND) and the mul-

tidrug and toxic compound extrusion (MATE) and ATP-binding cassette (ABC) in gram negative and gram positive bacteria

cell membrane is due to the synergistic effect between antibiotics and NPs. The combination of ZnO-NPs with antibiotics may form a complex that can disturb the cell membrane of *A. baumannii* and facilitate the uptake of antibacterial agents [63]. Another investigation showed that the susceptibility of *A. baumannii* increased after exposure to combination of ZnO-NPs and antibiotics. The study suggested that this phenomenon may be due to the enhancement of membrane permeability and blocking of efflux pumps in this bacteria [63]. Combinations of nanoparticles and the antibiotic are advocated to cope up the antibiotic resistance as the combinations are not only more active against pathogens but also decreases the toxic dosage of antibiotics and nanoparticles, when administered individually [66]. Figure 2 depicts the antimicrobial activity of zinc oxide nanoparticle.

4 Chitosan

Chitosan is a linear polysaccharide composed of randomly distributed β -(1 $\rightarrow$ 4)-linked D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acetylated unit) that is derived from chitin [67]. The polymer is biocompatible, non-toxic, eco-friendly, and a safe drug carrier. Encapsulation of the temporin B (TB) into chitosan nanoparticles (CS-NPs) have been shown to increase its antibacterial activity [68]. Chitosan has positive charge at acidic pH condition, therefore, it can damage the negatively charge cell wall of bacteria. In an investigation performed on mode of antimicrobial action of chitosan (polymeric beta-1,4-N-acetylglucosamine) on gram-negative bacteria it was observed that this nanoparticle rendered *E. coli* more sensitive to the

inhibitory action of dyes and bile acids used in selective media and on the other hand, highly cationic mutants of *S. typhimurium* were found more resistant to chitosan than the parent strains. Electron microscopy showed chitosan to cause extensive cell surface alterations and covered the outer membrane with vesicular structures, thus the NP appeared to bind to the outer membrane, explaining the loss of the barrier function [69]. In addition, chitosan does bind to DNA and inhibit the transcription and translation in microbial cells [34]. In year 2009, chitosan along with sulfamethoxazole was shown to reduce the minimum inhibitory concentration (MIC) value of sulfamethoxazole by fivefold in *P. aeruginosa* harboring a highly expressive MexEF-OprN efflux pump [70]. This result demonstrated that chitosan in combination with antibiotics can change expression of efflux pumps, although this findings further need some molecular investigations. Antibacterial activity of chitosan nanoparticle has been shown at Table 1.

5 Nitric oxide

Nitric oxide nanoparticles (NO NPs) uses several mechanisms to act on the bacteria. One of them is the production of reactive nitrogen oxide (RNOS) that can interact with bacterial proteins and eventually damages them. RNOS can also effect bacterial DNA and inhibit DNA repair enzymes. It interacts with zinc metalloproteinase and block respiration of bacteria [71] that cause decrease in efflux pumps activity. Earlier studies showed that action of NO NPs in concentration of 1.25–5 mM was enough to demonstrate antibacterial activity against MDR *E. faecalis*, *K. pneumoniae*, *E. coli*, *P. aeruginosa* and MRSA (methicillin

Table 1 Antibacterial activity of nitric oxide, chitosan and gold nanoparticles have been shown

Nanoparticles	Antibacterial activity	Bacteria	References
Nitric oxide	NONPs have broad spectrum significant antimicrobial activity acting against both Gram-positive and negative bacteria They releases nitric-oxide (NO) that can bind to proteins and lipids of plasma membrane and change their integrity and respiration chain of bacteria was damaged, so decreasing in efflux pumps was happened	MDR bacteria including: <i>E. faecalis</i> , <i>K. pneumoniae</i> , <i>E. coli</i> , <i>P. aeruginosa</i> and MRSA	[35, 71–73]
Chitosan	Amin group of glucosamine in chitosan cause positive charge of it that can effective on negatively charge cell membrane surface of bacteria	Destructive in gram negative bacteria more than gram positive bacteria	[132–134]
Gold	Gold have a few antibacterial effects, but in combination with 4, 6-Diamino-2 pyrimidinethiol, p-Mercaptobenzoic acid, mecapto ethylamine and 3-mercapto and propylsulfonate was increased against bacteria AuNPs can inhibit producing subunits of efflux pumps by inhibiting binding of tRNA to the ribosome by blocking translation phase and also decrease efflux pump by collapse of membrane and inhibit ATPase activities	Vancomycin-resistant enterococci (VRE), vancomycin resistant <i>E. coli</i> , <i>E. aerogenes</i> , <i>P. aeruginosa</i> and methicillin resistant isolate of <i>S. aureus</i>	[135–138]

resistant *S. aureus*; an MDR strain that over expresses efflux pump) [35, 72]. NONPs do have broad spectrum significant antimicrobial activity acting against both gram-positive and negative bacteria and these nanoparticles releases nitric-oxide (NO) that can bind to proteins and lipids of plasma membrane and change their integrity. NO can easily be transferred to the cytoplasm where it can interact with DNA, RNA and enzymes too [73]. Nonetheless, molecular study is necessary to understand NO NPs interactions with efflux pump. Antibacterial activity of nitric oxide nanoparticle has been shown in Table 1.

6 Magnesium oxide

Magnesium oxide nanoparticles (MgO NPs), another metal nanoparticle which stimulate ROS formation leading to the damage in the bacterial cell, is postulated to have a reverse effect on the efflux pumps of bacteria [34]. The antibiotic activity of MgO NPs increases after halogen molecules such as, Cl₂, Br₂ and F₂ are adsorbed to these nanoparticles. Magnesium chloride (MgCl₂), magnesium bromide (MgBr₂) and magnesium fluoride (MgF₂) nanoparticles have been documented to have bactericidal and anti-biofilm activity against *Bacillus subtilis* and *S. aureus* [34, 72, 74]. Figure 2 displays the antimicrobial activity of magnesium nanoparticles.

7 Copper

Comparative to other nanoparticles, these nanoparticles are less exploited. Diverse mechanisms of actions have been elucidated for copper including; direct interaction and permeabilization of the bacterial cell membrane, production of free radicals, RNA degradation, DNA strand breakage and cross linking, disordering DNA helical structure, DNA mutations, oxidation, mutation or cleavage of proteins, and displacement of essential metals of proteins [61, 75–81]. *Enterococcus faecalis*, *S. aureus* and *E. coli* are documented to be more susceptible to copper nanoparticles than *P. aeruginosa* [82]. Interestingly, copper nanoparticles have been demonstrated to act at various concentrations on different bacteria. For instance, the efflux pumps of *S. aureus* were observed to be inhibited at 0.032 mM concentration whereas, the efflux pumps of the wild type *P. aeruginosa* and *S. aureus* were highly inhibited at 0.065 mM concentrations [61]. Copper nanoparticles have been demonstrated to lower the MIC of the ciprofloxacin by functioning as an efflux pump inhibitor in MRSA [61]. Nor A is a predominant efflux pump in *S. aureus* that contributes to MDR phenotypes [21, 83] and copper nanoparticles inhibits the activity of Nor A. The

same research investigation showed copper nanoparticles to cause significant biofilm inhibition in both *P. aeruginosa* and *S. aureus* at lower concentration [61]. The reason of susceptibility of biofilms at lower concentrations is associated with multiple antibiotic response regulators (Mar R) which are copper sensors. In the lower concentration of copper, cysteine residues (cys80) of Mar R change to tetramerization by disulfide bonds, so the transcription of the biofilm is stopped [84]. Copper ions from nanoparticles might act as signaling molecules in *S. aureus* and change gene expression of exopolysaccharide molecules. Antibiofilm agents when used in combination with a number of efflux pump inhibitors significantly reduce biofilm formation and could even abolish biofilms in RND and MFS pumps expressing strains [82, 85]. Totally, efflux inhibitory and antibacterial effects of copper are partly mediated by copper (II) ions and partly mediated by copper nanoparticles. Antimicrobial activity of copper nanoparticle has been shown in Fig. 2.

8 Iron

Iron nanoparticle have been used as hyperthermia agents [86, 87], as carriers for targeted drug delivery to treat several types of cancer [88–90], as contrast agents for magnetic resonance imaging (MRI) [89, 91] and as bactericidal agents [92–95]. The bactericidal mechanisms of iron nanoparticle is due to the production of intracellular oxidative, such as OH, Fe(IV), generated by the reaction with hydrogen peroxide or other species [96], oxidative stress generated by reactive oxygen species (ROS) [96, 97], disruption of cell membrane integrity [93]. Bactericidal effects of iron nanoparticle under anaerobic conditions are greater than under air-saturated conditions [94]. Expulsion of rifampicin and other anti TB drugs in *M. smegmatis* are made effective by iron oxide nanoparticle covered with polyacrylic acid [98]. ROS generated by iron nanoparticle is the reason of damage of DNA and proteins in bacteria and probably impairment of the efflux pumps. The potential advantages of iron nanoparticle compared with others are easy preparation, low cost and high activity. Antimicrobial activity of iron nanoparticle has been shown at Fig. 2.

9 Calcium

Use of dental composites are growing due to their improved performance and esthetics. Dental composites consist of a polymerizable resin matrix, reinforcing glass particle fillers, and silane coupling agents which have good aesthetic properties and strength, making them the most widely used materials for the restorations of anterior teeth [99, 100] but

composites used in vivo show that they are limited to time and may lead to plaque and biofilm formation [100, 101]. Therefore, improving the longevity of composite restorations is needed. The composite restorations should incorporate bioactive agents to combat recurrent caries, microbial destruction and sustaining the load-bearing capability [102]. Various classes of antibacterial dental composites are used including, polymerase containing QAS (quaternary ammonium salts) [103, 104], silver (Ag) [102, 105], calcium phosphate (CaP) particles [106, 107]. The CaP composites releases supersaturating levels of phosphate (PO_4) and calcium ions and rematerialized tooth lesions [106, 107]. It has been demonstrated that the combination composites of calcium phosphate nanoparticle, QAS nanoparticle and silver nanoparticle greatly reduced the metabolic activity, biofilm growth, lactic acid production and colony forming units (CFU) counts of *S. mutans* compared with two other commercial composites [108]. In another study, when calcium hydroxide was used as an intracranial dressing, its bactericidal effect was found associated with its high pH (12.5–12.8) [109]. When an investigation was performed to evaluate the role of efflux pumps in altering the susceptibility of *Enterococcus faecalis* biofilms to calcium hydroxide [$\text{Ca}(\text{OH})_2$], chitosan nanoparticles, and light-activated disinfection (LAD), it was observed that *E. faecalis* biofilms were found to persist even after 24-h treatment with different concentrations of $\text{Ca}(\text{OH})_2$. On the other hand, LAD completely inactivated 4-day-old *E. faecalis* biofilms. The addition of EPI improved the antibiofilm efficacy of $\text{Ca}(\text{OH})_2$ at lower concentrations ($P < 0.001$), but had no effect on higher concentrations [110]. In contrast to the above findings, other researchers did not accept calcium hydroxide as an effective agent against *E. faecalis* in infected tooth models [111, 112].

Antibacterial effect of calcium hydroxide is associated with calcium ions. The binding sites of calcium ions on gram positive bacteria are phosphate and carboxylate groups on the cell surface [113, 114]. No study has yet investigated effect of calcium nanoparticles on efflux pumps.

The potential bactericidal effect of calcium hydroxide is associated with calcium ions that facilitate the diffusion of OH^- ions into the cell wall. Binding of calcium ions to the anionic groups, neutralize the anionic charges and reduce the repulsion of anionic entities of the cell wall [110]. Thus, the bactericidal mechanism of calcium nanoparticles may probably be due to the calcium ions released from the dissociation of calcium nanoparticles.

10 Platinum

Platinum is known to inactivate bacteria by interacting with their proteins, DNA and enzymes restraining cell division and cell proliferation [115]. Colloidal platinum

nanoparticles have been assessed for their ability to reduce the pulmonary and epidermal inflammations [116, 117]. As platinum oxide has less environmental pollution compared with other metals, thus the metal in the form of nanoparticle is considered a proper candidate to combat with environmental pathogens [118]. Platinum oxide stabilized with sucrose exhibited bactericidal activity against *Lactobacillus* species and *Pseudomonas stutzeri* [118]. Another investigation demonstrated that platinum nanoparticles with the 1–3 nm size exhibited bactericidal properties against clinical pathogen *P. aeruginosa* while the 4–21 nm exhibited bacterio-compatible properties in the same *P. aeruginosa* [119]. The anti-efflux pump effects of platinum have not been evaluated and the most studies of bactericidal effects of platinum nanoparticles have focused on the combination of platinum with other bactericidal components.

11 Gold

Gold nanoparticles are superior due to their controllable size, easily modifiable with desired molecules, chemically stable, and being nontoxic to mammalian cells and animals [120–123]. Gold nanoparticles have served as a flexible platform for exploring several aspects of basic sciences such as by linking to single molecules, conjugation with DNA, oligosaccharides, proteins or small bio functional molecules, finding applications in biology and assisting in fundamental developments in materials and physical science [89, 124]. Gold nanoparticles are an ideal model system to multivalency and polymeric based nanoparticles because they are 10^3 times smaller than bacterium (4–5 nm in diameter). In addition, they maintain a contrast shape and size in solution [125]. These nanoparticles have been found to enhance enhanced in vitro bactericidal activities against vancomycin-resistant *Enterococcus* (VRE), probably acting as a rigid polyvalent inhibitor [125]. Moreover, influence of gold nanoparticles conjugated to N-acetyl lactosamine have been observed to reduce the binding of EPEC (enteropathogenic *Escherichia coli*) to the epithelial cells and thus their localization [126]. Gold nanoparticles have been found to penetrate the cell wall of *Corynebacterium pseudo tuberculosis* and accumulate as intracellular agglomerates [127]. In an another study it was found that pyrimidine-presented on gold nanoparticles execute their antibiotic actions via sequestration of calcium and magnesium ions to disrupt the membrane of bacterial cells, resulting in the permeation of cytoplasmic contents such as nucleic acids and also act via inhibition of protein synthesis and interaction with DNA by internalized nanoparticles [128]. In the other studies

vancomycin-resistant Enterococci (VRE) were shown to be susceptible to gold nanoparticles coated with vancomycin [58, 125]. Ampicillin conjugated to gold nanoparticles was observed to be effective as broad-spectrum antibacterial agent acting against both gram positive and negative bacteria such as *E. aerogenes*, *P. aeruginosa* and MRSA while, gold nanoparticles uncapped with ampicillin had no activity against these bacteria [129]. Results of this study concluded that blockage of efflux pump and multivalent presentation of ampicillin are probably the causes of more effective action of ampicillin-capped gold nanoparticles compared with ampicillin alone. In another contemplation, antibacterial activities of AuNPs were seen on the *E. coli* concluding that these nanoparticles act by inhibiting tRNA binding to the subunit of ribosome, collapse of membrane

potential and impeding ATPase activities [128]. Attention paid by Zhao et al. from China and later Khameneh et al. from Iran to develop antibacterial nanodrugs so as to unwrap the antibiotic resistance revealed that gold NPs in combination with ampicillin had an effective antibacterial activity against high ampicillin resistance bacteria [32, 130]. They speculated that inhibition of the efflux pumps by the ampicillin bound to the gold NPs are the fundamental reason of the antibacterial effects of these agents. Despite these advances in the scientific world, gold nanoparticles alone could not erect the standards [32]. In search for new strategies to enhance antibacterial activity of antibiotics, the combination effect of gold materials including trivalent gold ions (Au^{3+}) and gold nanoparticles (Au NPs) with 14 different antibiotics was investigated against *P. aeruginosa*, *Staphylococcus*

Fig. 3 Ag NPs, ZnO NPs, MgO NPs, CuO NPs and Iron nanoparticles can damage DNA, mRNA, and peptide of bacteria by effecting on production of reactive oxygen species (ROS) (it was shown by black dotted arrows). In addition, Ag NPs can damage the cell membrane, DNA, mRNA, peptide production, electron transport chains and on the efflux pumps directly (it was shown by black continuous arrows). Iron nanoparticles can damage the cell membrane directly (was shown by orange continuous arrow) and have

indirectly effects on DNA, transcription and translation phases. ZnO NPs can damage the cell membrane and efflux pumps directly (was shown by purple continuous arrows) and has an indirect effect on DNA, mRNA and translation phase (was shown by dotted arrows). CuO NPs can damage the cell membrane directly (was shown by continuous arrow) and can effect on the translation and transcription phases indirectly

aureus and *Escherichia coli* clinical isolates by disk diffusion assay. They demonstrated that the susceptibility of resistant *P. aeruginosa* increased in the presence of Au3+ and methicillin, erythromycin, vancomycin, penicillin G, clindamycin and nalidixic acid, up to 147%. Parallel to this, similar results were observed when the same group of antibiotics were tested against *S. aureus*, *E. coli* clinical isolates and a different *P. aeruginosa* resistant strain in the presence of sub-inhibitory contents of Au3+, where Au3+ increased the susceptibility of test strains to methicillin, erythromycin, vancomycin, penicillin G, clindamycin and nalidixic acid. Their finding suggested that using the combination of sub-inhibitory concentrations of Au3+ and methicillin, erythromycin, nalidixic acid or vancomycin may be a promising new strategy for the treatment of highly resistant *P. aeruginosa* infections. However, more laboratory and molecular tests are required to be done in order to determine the effects of Au3+ and AuNPs in combination with antibiotics on *P. aeruginosa* cell wall permeability and their efflux pumps [131]. Antibacterial activity of gold nanoparticle has been shown in the Table 1 (Fig. 3).

12 Conclusion

Nanoparticles provide important antibacterial strategies to combat bacterial resistance. Recent studies mostly focused on in vitro effects of nanoparticles. Therefore, analysis of in vivo antibacterial activities and toxicity of nanoparticles may accelerate the realization of metal nanoparticles-enabled antibiotics. In addition, bactericidal mechanisms of metal nanoparticles have not been completely evaluated. In this review we concluded that some of the nanoparticles provide bactericidal effects and may have not anti-efflux pump effects such as platinum and calcium hydroxide whereas, some of them provide both bactericidal and anti-efflux pump effects such as chitosan, silver, gold, iron and copper. Their antibacterial activity and anti-efflux pumps effects increased after combination with antibiotics. Further molecular and proteomics study are required to realize effects of nanoparticles on different classes of efflux pumps in the resistant bacteria.

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Compliance with ethical standards

Conflict of interest No conflict of interest declared.

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