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Development of FDA-Approved Antibacterial Metal and Metal Oxide Nanoparticles: An Update

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Abstract

Nanotechnology is an emerging discipline for biomedical application. Nanoparticles (NPs) research is one of the most studied and rapidly evolving field with its wide range of diagnostic and therapeutic applications, particularly in antimicrobial development. Following the improvement of the biomaterial's functionality, the new area of 'nanocomposites' which often refers to the combination of NPs with other biomaterials such as hydrogel, polymers or other stabilizers, has swiftly followed. In the past decades, bacterial infections have caused negative impacts on human health, social and economic development in the globe. These problems are further aggravated by antibiotic resistance issues caused by drug-resistant microbes. With this, the development of antibacterial NPs has become an important field to alternate for the discovery of novel antibacterial agent. This review aims to discuss the key features of NPs, primarily derived from metal and metal oxide, for their antibacterial use in the clinic, the mechanisms of bacterial killing, and to cover some of the key challenges towards the Food and Drug Administration (FDA) approval for clinical use.

Keywords: *Drug, Antibacterial, Nanoparticles, Metal, Metal oxide.*

1. INRODUCTION

The development of nanotechnology has benefited various disciplines such as information technologies, engineering and construction, and health and medicine. Nanotechnology in medicine, also known as 'nanomedicine', has swiftly leapt forward and contributed to new discoveries, modification of drugs and medical devices. To date, the production of wound dressing, implantable stent and devices represents a large market of nano-based product in the healthcare industry [1]. Among the unique properties of nanomedicine compared to other bio-logically active materials or compounds are their nano-scale size (1-100nm) and shape which makes them suitable to act either alone as an efficient cell-penetrating therapeutic agent or as nanocarrier to transport desired drugs to the target. More importantly, nano-based products have rich surface chemistry such as large surface

area to volume ratio and high compatibility with diverse ligands and functional groups which allows them to be highly modifiable or conjugated with any bioactive nano-materials [2]. Since 1990, nanomaterial-based drugs have been approved by European Medicines Agency (EMA), Food and Drug Administration (FDA), or other authorized institutions for clinical use [3]. Table 1 summarizes the nanomaterial-based drugs approved for various clinical applications, including anticancer, antiviral, and antimicrobial actions in the last decade.

Table 1. *Approved nanomaterial-based drugs from 2011 to 2021.*

Brand name	Nanoparticle/ loaded drugs	Clinical application	Approval year	Agency
Exparel®	Bupivacaine	Pain management	2011	FDA
Nanoxel® M	Docetaxel	MBC, NSCLC, and ovarian cancer	2012	MFDS
Marqibo®	Vincristine	ALL	2012	FDA
Injectafer®	Iron nanoparticles	Iron deficiency anemia	2013	FDA
Adynovate®	Polymer-protein conjugate	Hemophilia A	2015	FDA
Paclical®	PTX	Ovarian cancer	2015	RFMPH
Invega Trinza®	Paliperidone palmitate	Schizophrenia	2015	FDA
Onivyde™	Irinotecan	Metastatic pancreatic cancer	2015	FDA
Vyxeos®	Daunorubicin and Cytarabine	AML-MRC and t-AML	2017	FDA
Shingrix®	Recombinant VZV glycoprotein E	Against shingles and post-herpetic neuralgia	2017	FDA
Aristada Initio®	Aripiprazole lauroxil	Schizophrenia	2018	FDA
Onpattro™	siRNA	Polyneuropathy caused by hATTR	2018	FDA
Arikayce® Kit	Amikacin	NTM lung disease caused by MAC	2018	FDA
Invega Hafyera®	Paliperidone palmitate	Schizophrenia	2021	FDA
Cabenuva®	Cabotegravir	HIV-1 infection	2021	FDA
Apretude®	Cabotegravir	HIV-1 infection	2021	FDA
Mosquirix®	Recombinant CSP	Malaria	2021	EMA
Comirnaty®	BNT162b2	COVID-19	2021	FDA
mRNA-1273	mRNA-1273	COVID-19	2021	FDA

ALL- Acute lymphoblastic leukemia; AML– Acute myeloid leukemia; AML-MRC- AML-myelodysplasia-related changes; FDA– U.S. Food and Drug Administration; hATTR- hereditary transthyretin amyloidosis; MAC- Mycobacterium avium complex; MBC- Metastatic breast cancer; MFDS- Ministration of Food and Drug Safety; NSCLC- Non-small cell lung cancer; NTM- nontuberculosis mycobacteria; RFMPH- Russian Federation: Ministry of Public Health; PTX- Paclitaxel; VZV- varicella-zoster virus.

Amongst the countless of biological activities exerted by nanoparticles (NPs), their antibacterial properties remain the most extensively studied field and new discoveries of novel antibacterial NPs is ceaselessly in search. Although bacterial infections are not as deadly as other non-communicable diseases such as cardiovascular diseases and cancers, according to the world statistics [4], the spread of the bacterial infection can result in a negative social and economic impact, and exorbitant healthcare cost in the long run. These burdens will certainly be escalated due to the emerging antimicrobial resistance (AMR) which is a global issue that arose from the overuse and misuse of antimicrobial drugs [5]. Hence, other non-antibiotic alternatives are being sought to look for novel antibacterial agents that are less susceptible to resistance [6]. Undoubtedly, NPs or other nanomaterials with broad biological and pharmaceutical values are one of the prioritized agents for research and further evaluation. In the last decade, multibillions have been invested in the global market of nanomedicine for both diagnostics and therapeutics purpose including those targeting bacterial infections [7].

Very recently, several key reviews have discussed and updated on the development of antibacterial NPs for potential clinical uses. For examples, Hetta *et al.* [8] comprehensively covered the strategies of adopting the nanomaterials to combat against multidrug-resistant (MDR) bacteria while Geng *et al.* [9] highlighted several possibilities in modifying the antibacterial NPs for their improved therapeutic efficacies. Due to the fast-growing discipline, there is a need to revisit the current development of NPs for antibacterial use, particularly those derived from metal and metal oxide which accounts for the largest group of NPs.

Moreover, the rise of antimicrobial drug resistance pathogens has been declared as one of the global health threats by the World Health Organization (WHO) in the 21th century [10]. Due to the high efficacy of NPs against microbes, NPs have become the subject of interest in the fight against antimicrobial drug resistance pathogens [8, 11]. Metal NPs such as silver NPs (Ag NPs) and gold NPs (Au NPs) and metal oxide NPs like copper oxide NPs (CuO NPs), Zinc oxide NPs (ZnO NPs) and titanium oxide NPs (TiO NPs) have shown potent antibacterial actions against several MDR pathogens [12]. Silver NP is another NP that has shown potent antimicrobial effect against MDR bacteria [13, 14]. For example, silver NPs destabilized the bacterial oxidation process, resulting in a failure to remove excess reactive oxygen species (ROS), hence killing MDR *Pseudomonas aeruginosa* by accelerating ROS production [15, 16]. CuO NPs has also shown antibacterial effect against several MDR uropathogenic bacteria such as *Enterococcus faecalis* [17].

In this review, emphasis is focused on the antibacterial properties of metal and metal oxides NPs, as well as their characteristics such as physical morphology, surface chemistry and their controlled release by stimuli which influences their antibacterial actions. Various antibacterial mechanisms such as membrane perturbation, pore formation in cell wall, biofilm inhibition and reactive oxygen species (ROS) formation of the metal and metal oxide NPs are also explored. In addition, several existing FDA-approved NPs and NPs under clinical trials together with their antibacterial applications are discussed, followed by limitations and key challenges in developing the new NPs towards FDA approval.

2. ANTIBACTERIAL METAL AND METAL OXIDE NPS

2.1. Metal Nanoparticles

Inorganic NPs such as metal and metal oxide remain the most studied NPs for various biomedical applications. Metallic NPs derived from gold (Au), silver (Ag),

copper (Cu), platinum (Pt), iron (Fe), and bimetallic (*e.g.*, combination of Ag and Au) have shown promising antibacterial actions against a wide range of bacteria in the past, Au and Ag NPs being the largest groups of antibacterial NPs. These metallic NPs can be synthesized by conventional chemical method or through 'green' method, which is drawing much attention in the recent years. Biosynthesis using 'green' materials have several advantages over the traditional method such as lower cyto-toxicity, more environmentally friendly and lower production cost. There are several key reviews which have summarized the potent antibacterial activity of AuNPs [18], AgNPs [19] and CuNPs [20] against both Gram-positive and negative bacteria.

There are many reports suggesting the mechanisms of these NPs, including cell wall disruption, ROS production and decomposition of cellular components, some of these NPs were found to exhibit even higher antibacterial activity than antibiotics. For examples, a study by Esmaeillou *et al.* reported that AgNPs exhibited lower minimum inhibitory concentration (MIC) compared to vancomycin when treated against *Staphylococcus aureus, Enterococcus faecalis, S. epidermidis, Pseudomonas aeruginosa* and *E. coli* [21]. Certain metallic NPs even showed promising bactericidal action against MDR bacteria such as methicillin-resistant *Staphylo-coccus aureus* (MRSA), Vancomycin-resistant Enterococci (VRE), ampicillin-resistant *E. coli*, and erythromycin-resistant *Streptococcus pyogenes* [22,23]. Accumulating evidences also showed that the combination of metallic NPs with antibiotics could significantly enhance the antibacterial potency. For example, in a study by Li *et al.*, a synergistic antibacterial effect of AgNPs was observed in *E. coli* when combined with β-lactam antibiotic, amoxicillin [24]. The study also showed that the combination of AgNPs and amoxicillin exerted a greater bactericidal effect against *E. coli* when compared to when it was treated separately. In another research conducted by Murugan and Paupandian, they investigated the antibacterial effect of FeNPs as well as FeNPs when combined with other antibiotics [25]. Although when treating bacterial strain with FeNPs alone showed antibacterial activity with the antibacterial effects varying across the different bacterial species, they also observed a significant increase of antibacterial effect which was observed when antibiotic such as Vancomycin, Tetracycline, Streptomycin, Chloramphen-icol, Ampicillin, and Erythromycin were combined with FeNPs and treated against *S. epidermidis, B. cereus, Pseudomonas aeruginosa, E. coli, K. pneumoniae* and *S. aureus* with an overall synergistic bactericidal effect of 29%, 28.58%, 16.07%, 21.99%, 29.75% and 24.71% in each bacterial species respectively. Kiranmai *et al*. also studied the synergistic antibacterial effect of biologically synthesized copper NPs from green tea (*Camellia sinensis*) [26]. It was observed that the antibacterial effects of Ampicillin, Amoxicillin, Gentamicin and Cipro-floxacin were greatly enhanced when combined with the copper NPs against both Gram-positive and Gram-negative bacteria. They observed an overall synergistic activity of 24.94% when combined with Ampicillin, 5.97% when combined with Amoxicillin, 6.23% when combined with Gentamicin and 17.34% when combined with Ciprofloxacin.

Moreover, the synthesis of bimetallic NPs could also synergistically result in bacterial killing, hence significantly reducing the active dose and cytotoxic effects. For example, in a study by Fakhri *et al*., they studied the antibacterial effect of silver- gold bimetallic NPs and their synergistic effect when combined with the antibiotic, Doxycycline against *P. aeruginosa, E. coli, S. aureus* and *M. luteus* [27]. They found that combining the bimetallic NPs and the antibiotic exhibit a

greater bactericidal effect compared to their lone counterpart. Nazeruddin *et al.* studied the antimicrobial effect of the bimetallic NPs, silver-copper NPs against pathogenic bacteria, *B. subtilis and S. typhimurium* [28]. Again, synergistic antimicrobial effect was observed from the silver-copper bimetallic NPs. In another study by Parimaladevi *et al*, they synthesized copper-nickel bimetallic NPs and found that they increased antibacterial synergistic effect when combined with the drug cefixime, when treated against *E. coli* and *S. aureus* as compared their lone counterpart [29].

2.2. Metal Oxide Nanoparticles

As mentioned earlier, metal oxides NPs are also one of the extensively studied NPs for antimicrobial applications. Some of the examples are iron oxide (Fe_3O_4), zinc oxide (ZnO) and copper oxide (CuO) NPs [30,31]. Similar to metallic NPs, Fe_3O_4 NPs exerted promising inhibition against a wide range of bacterial strains. For instance, biosynthesis of iron oxide NPs using *L. siceraria* leaf extract exhibited antimicrobial activity on *E. coli* and *S. aureus* [32]. One of the unique properties about Fe_3O_4 NPs is that it possesses superparamagnetic properties which allows them to be directed to specific infected area without affecting other parts of the organism by applying an external magnetic field and by modifying the surface functionality with an antimicrobial agent that can be used in antibacterial treatment which requires specific targeting by using a magnetic system [33]. A study was designed to test the antibacterial effect of superparamagnetic iron oxide NPs (SPIONs) against *S. mutans*. The SPIONs effects were subjected to the bacteria and their biofilm which they concluded that that SPIONs showed toxicity against bacteria that was incubated in solution and against their biofilms [34].

Metal oxide NPs have been shown to be broad spectrum, exhibiting antibacterial effects against both gram-positive and gram-negative bacteria and MDR bacteria. Ravikumar *et al*. investigated the antibacterial effect of five metal oxide NP namely, aluminium oxide (Al_2O_3), iron (III) oxide (Fe_2O_3), cerium (IV) oxide (CeO_2), zirconium dioxide (ZrO_2) and magnesium oxide (MgO) were tested against *Pseudomonas sp., Enterobacter sp., Klebsiella sp., E. coli, Proteus morganii* and *Staphylococcus aureus*, which mainly constitute of infectious pathogens found in the urinary tract [35]. They found that all the NPs showed sensitivity against all the pathogens except against *Pseudomonas sp*. However, Al_2O_3 NPs exhibit better and higher antibacterial effect against the infectious pathogens.

Combination of metal oxide NPs with antibiotics and other pharmaceuticals has also proven to enhance the antibacterial activity even while lowering the active concentrations of drugs. The antibacterial effect of zinc oxide NPs combined with Cefotaxime, Ampicillin, Ceftriaxone and, Cefepime were tested against *E. coli, K. pneumoniae, P. aeruginosa* and *S. paucimobilis* which are all β-lactamase producer strains and it was found that the antibacterial effect of the antibiotics were greatly enhanced in the present of Zinc oxide which facilitates the damage of the cell membrane by the β-lactam antibiotics where the dynamic time-kill curve of the bactericidal activities was seen to have increased from 50% to 85% fold depending on the bacteria species indicating synergism between Zinc oxide NPs and the β-lactam antibiotics [36].

The mechanism of bacterial killing is mainly attributed to the dissolution of metal ions and the generation of ROS. Titanium dioxide (TiO_2), copper oxide (CuO), and zinc oxide (ZnO) are ROS production metal oxide NPs. Those metal oxides can induce production of ROS either extracellularly or intracellularly. For example, if there is an accumulation of ROS extracellularly or on the cell surface of the bacteria, then membrane leakage may occur and if there is an accumulation

of ROS intracellularly, leakage through the membrane, alteration of protein and damage to the DNA may occur [37]. Other mechanisms such as biofilm inhibition and cell wall perturbation have also been described. Interaction of metal NPs and metal oxide NPs with bacterial cell surface showed physical and chemical induction of toxicity. This is mainly due to the surface charge of the NPs and their shape which gives the NPs unique abilities to interact with the bacteria cell surface, transport into the cell and ultimately causes damage either physically or chemically to the cell surface [37, 38].

The fast-growing development of biomaterial science has boosted the antibacterial application using NPs in combination with other biocompatible materials, known as 'nanocomposite'. These biomaterials act slightly different than the concept of nanocarriers, as they usually serve as stabilizer or reducing agent for the NPs and incorporated into the NPs to form the nanocomposites [39]. Some of the examples are polymer-stabilized NPs and biomolecules-functionalized NPs. Other than being used for antimicrobial application alone, NPs may also serve as nanocarrier to deliver active compounds to the bacterial targets. Due to the rich surface chemistry, NPs can be modified to transport a vast range of both organic and inorganic cargoes to the targets. They can be modified into many forms such as antibiotics-capped NPs, mesoporous silica NPs, liposomal NPs, micelle NPs, protein NPs nanocrystal NPs, dendrimer NPs and polysaccharides NPs [40].

3. CHARACTERISTICS CONTRIBUTING TO ANTIBACTERIAL NPS

3.1. Size and Shape

One of the important properties of NPs is their nanoscale sizes, typically 1-100 nm. This advantage allows NPs to effectively kill a wide range of pathogenic microorganisms such as fungi, bacteria and even viruses. Generally, the diameter of viruses may vary from the smallest being 20 nm up to 400 nm for the largest viruses [41]. NPs being in the nanoscale range, may exert potent effects against viruses. For example, studies have shown that silver NPs possess antiviral effect against viruses such as hepatitis B virus, monkey pox virus, herpes simplex virus, human immunodeficiency virus, and respiratory syncytial virus. This may be because of the property of metal NPs that attack the viruses using different targets and as they are nanosized, such an effect could be enhanced [42]. For antibacterial application, it has been shown that the difference in sizes has a direct impact on its therapeutic efficiency against different bacteria. In a study conducted by Azam *et al.,* different sizes of Copper oxide NPs were synthesized using gel combustion, where portions of the samples were annealed at different temperatures of 400˚C, 500˚C, 600˚C and 700˚C to synthesize varying sizes of Copper oxide [43]. The differently sized NPs were tested against gram-positive and gram-negative bacteria, namely, *E. coli, P. aeruginosa, Staphylococcus aureus* and *B. subtilis*. Interestingly, copper oxide NPs showed antibacterial effect against the aforementioned microorganisms tested. Moreover, the Copper oxide NPs of the size 20 nm (the smallest size) showed greater antibacterial effect compared to the other sizes. In another study conducted by Dong *et al.,* different sizes of 10±5 nm, 30±5 nm, 60±5 nm, 90±5 nm in dimension of silver NPs was treated against *Vibrio natriegens.* Although, silver NPs at different sizes showed effective results against the bacteria, smaller sized silver NPs exhibited lower minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC), suggesting a greater potent antibacterial activity compared to bigger sized silver NPs [44]. This may be because smaller NPs are more potent due to their increased surface area and ability to effectively penetrate bacterial biofilm matrix and cell

membrane, leading to enhanced toxicity towards bacteria. Nevertheless, smaller sized NPs are more prone to agglomeration which may affect their dispersion stability [44]. Hence, the ideal size range of the NPs will depend on the intended clinical use and mechanism of action of the NPs. To ensure consistent and predictable performance of NP-based therapy, narrow size distribution of NPs ought to be achieved during the synthesis process.

Another unique feature of NPs is their shape. Similar to the effect NPs' sizes on antibacterial potency, different shapes also result in varied therapeutic efficacies against bacteria. The traditional way to manufacture NPs is using the bottom-techniques and the shape that the NP takes the shape of sphere. This is usually due to limiting factors such as minimal thermodynamic energy, restricted entropy and molecule self-assemble limitation, therefore NPs take a spherical shape to become more stable in these conditions as in this form, the interfacial energy of the NP is minimized due to lowered surface per unit volume. However, with the use of advanced technology such as Particle replication in non-wetting template (PRINT®), stretching spherical particle, and step and flash imprint lithography, unique shapes such as cube, rod, circular disc, cone, hexagon, elliptical disc oblate/prolate ellipsoid worm/long worm shape, triangular or pentagon cylinder, bullet or biconcave shape of NPs can be produced [41].

To test the effect of NPs shapes against bacteria. Cheon *et al.* synthesized three differently shaped silver NPs, namely, spherical, disk, and triangular plate silver NPs [45]. The various shaped NPs were treated against *E. coli, S. aureus* and *P. aeruginosa* to test their antibacterial properties. They found that spherical silver NPs are more effective in inhibiting microbial activity followed by disked-shaped silver NPs and finally the triangular plate silver NPs. They also discovered that the rate of release for silver NPs is the cause for the antibacterial properties of silver NPs and that their antibacterial properties are also closely related to the shapes of the NPs which possess different surface areas for silver ion release [45]. An explanation for this may be due to the amount of exposed plane surfaces and the level of oxidation of the metals, whereby the more unstable the plane surface is, the easier the production of oxygen species, thereby associating the stability of the plane surface of a specific NP shape to its antibacterial properties and production of ROS [46]. Therefore, it is important to take into account the shape of the NPs during the developmental phase as their geometric shapes play a role in their bioavailability, uptake into bacterial cells and antimicrobial activity, with spherical NPs possessing a competitive advantage over NPs of other shapes [45, 47].

3.2. Surface Chemistry, Solubility and Stability

NPs are known for their rich surface chemistry which allows high degree of modifications. This unique feature serves as the basis for developing novel NPs-based nanocarriers or nanocomposites. The surface chemistry of NPs usually decides the type of protein that can bind and interact with the NPs along with their strength of the interaction which affects the stability and solubility of the NPs. Most NPs when delivered to the bloodstream would usually be taken up withing hours or even minutes by phagocytic cells. Nevertheless, the bioavailability of NPs can be prolonged by adding poly (ethylene) glycol (PEG) onto the surface of NPs to prevent opsonization and thus increasing the half-life of NPs [47]. Other protective agents that are used in the manufacturing of NPs are citrate and thiols. The properties of these NPs can be enhanced by using polymers, polypeptide, glycosides, and proteins, as solely using citrate and triols does not yield desirable results. However, the use of polymers, polypeptide, glycosides and proteins are

far from being produced in a large scale in the commercialization of NPs [48]. A study performed by Kvítek *et al.* described the dispersion, aggregation stability effect and antibacterial effect of different surface modification on silver NPs, using surfactants including ionic surfactant sodium dodecyl sulphate (SDS), the non-ionic surfactants Tween 80, Brij 35, 58, 97, and 98, and polymers PEG 1500, 4000, 1000, and 35000; Polyvinylpyrrolidone (PVP) 10, 40, and 360 [49]. The stability of the NP was evaluated using the titration method by the aqueous dispersion of the silver NP in aqueous solution of poly (diallyl dimethylammonium) chloride (PDDA). The aggregation effect of the NPs was measured using dynamic light scattering (DLS) and UV/vis spectra measurements and TEM. Among the ligands that was used to modify the surface of the silver NPs, SDS showed the highest stability during dispersion and aggregation process followed by Tween-80, and PVP 360. For the antibacterial effect study, SDS, Tween-80, and PVP 360 modified silver NP and unmodified silver NP was used against an array of bacteria such as *Enterococcus faecalis, E. coli, P. aeruginosa, S. aureus, S. epidermidis* (methicillin-susceptible), *S. epidermidis* (methicillin-resistant), Methicillin-resistant *S. aureus,* vancomycin-resistant *E. faecium,* extended-spectrum β-lactamases *K. pneumoniae*. The study concluded that SDS modified silver NPs demonstrated the highest antibacterial effect with the remaining modified silver NPs demonstrating enhanced antibacterial activities. The antibacterial effect of the SDS modified silver NPs was said to be related to their dispersion stability, solubility and the high interaction with the bacteria cell surface membrane.

3.3. Stimuli-Responsive Nanocarriers

In order to control the release of NPs, some NPs can be stimulated by various stimuli such as light, pH or heat. These manipulation strategies are particularly useful for nanocarriers which require highly specific target delivery of therapeutic agents. Extensive research has been conducted on the photocatalytic antibacterial properties of metal NPs, particularly on titanium dioxide NPs which have shown effective antimicrobial activity in which titanium dioxide NPs demonstrated the ability to inactivate bacteria such as *Enterococcus faecalis, Lactobacillus acidophilus, Bacillus subtilis, Micrococcus luteus, E. coli* and *S. aureus* in the presence of ultraviolet light. Zinc oxide and silver oxide are other metal oxide NPs that showed photocatalytic antibacterial effect against bacteria. It is agreed that ROS is produced by the metal oxide NPs upon exposure to ultraviolet light. Among these metal oxide NPs, ultraviolet exposed titanium oxide NPs exhibited great antibacterial potential [50].

A study by Pourali *et al.* demonstrated the effect of heat in relation with the antibacterial effect of silver NPs [51]. In this experiment, three different temperatures of the silver NPs (room temperature, 100˚C and 300˚C) were prepared respectively. The silver NPs were tested against *K. pneumonia, S. aureus, S. dysenteriae, S. epidermidis, E. faecium, L. monocytogenes, P. mirabilis, Y. enterocolitica, P. aeruginosa* and *E. coli*. According to the study, silver NPs that was not heated showed better antibacterial effect compared to silver NPs that were heated at 100˚C and 300˚C even though there was some activity due to the heated silver NPs sizes. It was hypothesized that the heat lowered the penetrative ability of NPs to enter the bacterial cells and induced the loss of extra cellular protein, known as capping agents.

An experiment conducted by Alpaslan *et al.,* showed the effect of changes in pH in relation to the antibacterial activity of cerium oxide NPs [52]. The study was performed on *P. aeruginosa* and *S. epidermidis* with varying pH of the NPs solution. It was found that the cerium oxide NPs were more effective at

eliminating both bacteria at pH 9 which is considered to be basic, compared to be to the acidic NPs solution of pH 6. This is because in basic solution the small size and the positive charge on the surface of the NPs enhances its antibacterial effect and the treatment with cerium oxide NPs results in the production of ROS.

Iron oxide NPs possess super-paramagnetic effect compared to their bulk counterparts. In a study performed by Javanbakht *et al*., the antibacterial effect of superparamagnetic iron oxide NPs (SPIONs) was tested against the biofilm and the aqueous solution of the *Streptococcus mutans* bacteria [34]. For this experiment, iron oxide NPs without any surface charge were used along with positively charged iron oxide NPs and negatively charged NPs. Iron oxide NPs were used to evaluate the super-paramagnetic effect of the NPs in relation with its antibacterial effect. It was found that the predominant functional group in the positively charged SPIONs was amine group whereas in the negatively charged SPIONs, the predominate functional group was carboxylic group. From the experiment, it was found that at low concentration, the positively charged SPIONs and the bare Iron oxide NPs have the same activity. However, at higher concentrations, the positively charged SPIONs showed higher bactericidal activity and it also showed that the positively charged SPIONs have a better bactericidal effect than the negatively charged SPIONs. However, when incubated into biofilm of the bacteria, all of the SPIONs types became more negative in the negatively charged biofilm.

This showed that the surface charge of the SPIONs is important in determining the extent of the bactericidal effect, but the surface charge does not stay constant and eventually have the same charge when incubated with the biofilm. Nonetheless, the study showed that the surface charge of the SPIONs is an important factor for the bactericidal effect whether against the aqueous solution of the bacteria or the biofilm.

4. ANTIBACTERIAL MECHANISMS OF METAL AND METAL OXIDE NPs

There are several major bactericidal mechanisms that has been suggested on how metal and metal oxide NPs antibacterial activity works. Among them is the production of ROS which results in oxidative stress which in turn causes DNA damage and lipid peroxidation. The other two mechanisms of action are the reaction of dissolved metal ion with the bacterial cellular components such as the cell membrane, and physical damage to the cell envelope or membrane (Figure 1).

The production of ROS usually occurs when the bacteria is subjected to intensive physical or chemical stress or toxicity which can be caused when treated with metal or metal oxide NPs such as silver NPs [53] and copper oxide NPs [54]. ROS is usually a by-product of aerobic activity in the bacteria cells and can act a regulatory compound [55]. Normally, bacterial cells are equipped with several pathways to limit the build-up of ROS [55]. However, when these pathways are expended, there is a loss in cellular functions and an increase in the concentration of ROS which becomes detrimental to the bacterial cells. Production of ROS can be induced both extracellularly by metal or metal oxide NPs and intracellularly when the NPs enter the cells and interact with biomolecules inside the bacterial cytoplasm [37]. Extracellularly, the ROS will interact with the cell membrane or cell wall of the bacterial cell where the ROS would induce membrane leakage by modification of the protein and lipid peroxidation [56]. Inside the cell, ROS causes the same effect as it would extracellularly and ROS will cause DNA damage and alter the functionality of the electron transport chain and protein pump [57, 58].

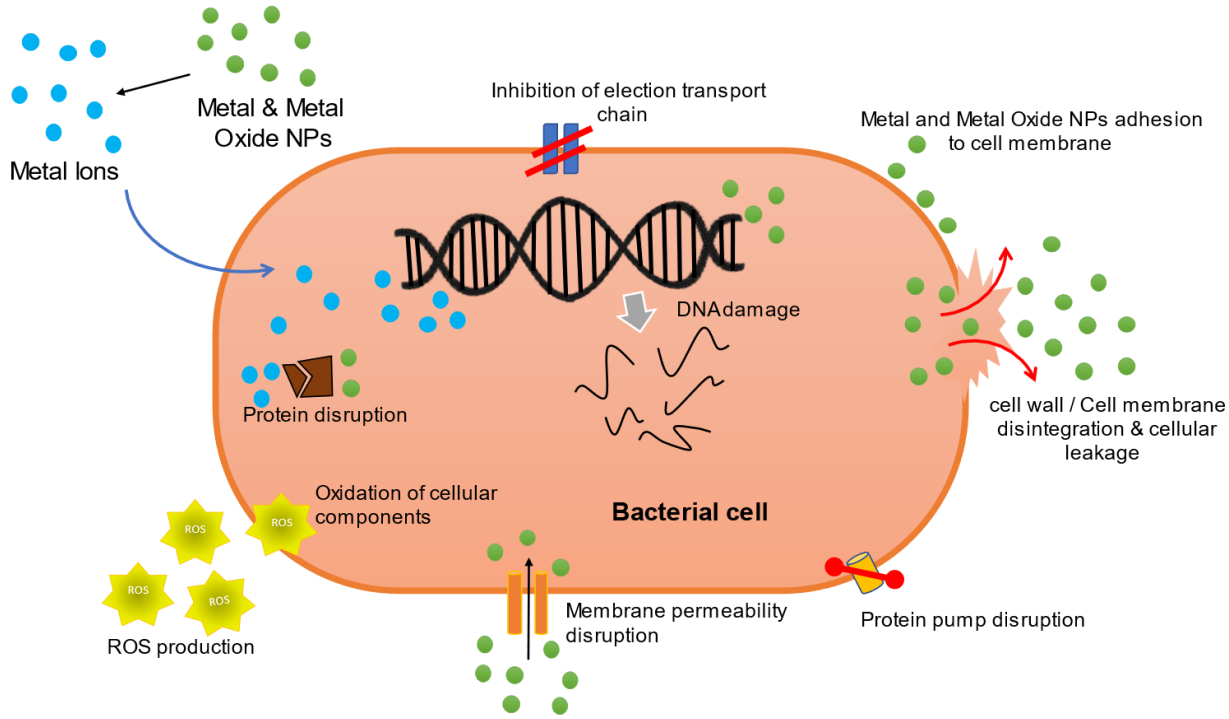

Figure 1. *Schematic illustration of the antibacterial mechanism of action of both metal and metal oxide NPs.*

Dissolved metal ions from metal NPs, such as silver NPs [59], and from metal oxide NPs, such as copper oxide [60] or zinc oxide [61] NPs, are usually responsible for the antibacterial effect demonstrated from the NPs. The dissolution of the metal ion from the metal and metal oxide NPs usually happens at the cell surface outside the cell or inside the cells when the NPs has been taken up. Irregular cell functions that occur from dissolved metal will lead to discoordination of cellular function and non-homeostasis [62]. Extracellularly or intracellularly, the metal ions would chelate to chemical entities which forms part of ligands such as oxygen, nitrogen, sulfur and phosphorus and carboxyl function groups which would alter the cellular structure and the functions of enzyme [63]. For example, silver ion from silver NPs [64] interacts with DNA, membrane protein and respiratory enzyme via thiol group of these biomolecules where the cellular functions are impaired [37, 65].

Local chemistry such as surface coating of the NPs, the compositions of lipopolysaccharides, strength of ionic interactions and pH are factors that helps metal and metal oxides to interact with the lipopolysaccharide membrane of gram-negative bacteria and the outer cell membrane or cell wall of bacteria inducing physical and chemical toxicity [66] and cellular leakage. Disruption of the membrane also occurs when adsorption of the metal and metal oxide NPs takes places. As the NPs are being adsorbed, the cell wall depolarizes and the disintegration of the cell wall occurs [67]. As mentioned above, surface coating is used to render the NPs more stable by introducing surface charge which can then interact with the membrane surface of the bacteria and thus interact with the bacteria. From there, the NPs can penetrate the cell causing chemical reactions [37]. Shape of NPs is also an important factor to consider for the disruption of bacterial cell envelope. Another example is zinc oxide nanorod, where the sharp edge helps in damaging

and causing physical stress to the cell membrane due to its structural features [66].

5. EFFECTS OF NPS ON HUMAN NORMAL CELLS AND NORMAL FLORA

Because of their wide usage, the potential negative impact on human health is of great concern [67]. The characteristics that give NPs their antibacterial properties might be responsible of their cytotoxicity in human normal cells [68,69]. For most of the biomedical application of NPs, the main point of contact in the body system is through the skin, respiratory and gastrointestinal tracts. NPs may also damage brain, heart and lungs [70]. For example, silver NPs coated with polyvinylpyrrolidone (PVP-AgNPs) was found to cause permanent alteration of gene and DNA damage in cells across several tissues in mice [71]. It was found that the inhaled zinc oxide NPs deposited within the lungs, attached to epithelia, and caused inflammation. The zinc oxide NPs also interacted with the entrance of interstitium, migrated to lymphatic nodes, and caused chronic effects to cells [71].

As NPs could exert potent antibacterial activity against a wide range of bacteria, it is not surprisingly that they could equally kill human normal flora. This may raise a concern as normal flora such as human gut microbiota plays important role in maintaining overall gut health including regulating gut mobility, gastric secretion, mucosal blood flow and permeability [72]. An imbalance in the gut microbiota, also known as dysbiosis, is associated with irritable bowel syndrome (IBS), inflammatory bowel disease (IBD) and metabolic syndrome [73]. Hence, the understanding on the effects of these normal flora is crucial. The interaction of the most commonly studied NPs, silver NPs, with human and animal gut microbiota both *in vitro and in vivo* has been previously reviewed [74]. It was found that mucosal layer on the gut lining prevents direct absorption of the NPs through intestinal cells, hence permitting longer exposure time of silver NPs to gut bacteria. This causes an imbalance in the gut microbiota population, increasing the chances to develop certain diseases [74]. For examples, metal oxides used in the food industry such as titanium dioxide (TiO_2), silicon dioxide (SiO_2) and zinc oxide (ZnO) NPs have shown their capacity to alter the composition of the gut microbiota. Having said that, other NPs such as iron oxide NPs (Fe_2O_3) have shown beneficial effect to the gut microbiota by increasing the diversity of population such as *Lactobacillus*, and this significantly enhances the overall gut health [75, 76].

6. QUALITY AND SAFETY CONTROL OF NPS

As abovementioned, some NPs may present detrimental effects to human cells and normal flora as well as environments. Therefore, standardized guidelines or regulation should be in place with regards to NPs synthesis especially at larger scale and their application should be closely monitored and controlled. However, there is very limited regulatory guidance in this area. This is largely due to the huge diversity of nanomaterials whereby the same procedure for assessing one nanoparticle may not be appropriate to assess another nanomaterial due to the differences in characteristics and functions. Currently, US FDA, Environmental Protection Agency (EPA) and European Commission Directorate-General for Health and Consumer Protection are involved in the management of nanomaterial risks [77]. Although the requirement for approval is ambiguous, they seem to align with the regulation for small drugs molecule [78]. Furthermore, REFINE which is a Regulatory Science Framework for Nanomaterial partnered with the National Institute for Public Health and Environment, Joint Research Centre (JCR) European Commission, and

European Research Services to define the regulatory criteria and improve the regulation of nanomedicines and nanomaterials for biomedical uses in a case-by-case study. The framework consists of optimal formulation, clinical trial and product approval. In optimal formulation, the main purpose is to understand the physio-chemical properties of the nanomaterials and their potential for pre-clinical trial, as well as their attributes during research and development (R&D) phase. The clinical trial phase includes their response during absorption, distribution, metabolism and excretion (ADME), pharmacokinetics and pharmacodynamic, compatibility and safety. The product approval phase consists of product monitoring, patient management and pharmacovigilance [79]. However, this framework is not being used universally worldwide and the regulation for nanomaterial across different regulatory agencies and countries are unclear, not standardized and inconsistent. This has significantly delayed the progress of developing the highly potential NPs for clinical use [77.79].

7. ANTIBACTERIAL NPS TOWARDS FDA APPROVAL

With a wide range of pharmacological and biological activities, countless NPs have been approved by FDA or EMA for clinical use. This includes the first FDA-approved nanodrug, Doxil made from PEGylated liposome loaded with doxorubicin, targeting cancers such as ovarian cancer, multiple myeloma, and HIV-associated Kaposi's sarcoma [81]. There are many more NPs targeting different types of diseases that are currently undergoing various trial phases for approval. In this section, we will focus mainly on NPs used for antimicrobial application.

With the gradual rise of antibiotic resistance strains, alternative treatments are being explored. It is widely known that silver is used to treat infections as it has mild toxicity towards human body compared to its increased toxicity towards bacteria, fungus, and virus [82, 83]. Therefore, Zhou *et al* developed a novel theragnostic system made up silicon 2,3-napthalocyanine dihydroxide (Nc) and Vancomycin functionalized silica-encapsulated silver-coated gold NPs (Au@AgNP@SiO_2@Nc-Van) [84]. The theragnostic ability of the silver-coated gold NPs are through surface-enhanced Raman scattering (SERS) and the antimicrobial photodynamic therapy towards vanco-mycin resistant *Enterococci* strains [85].

A 12-month study was conducted on children in poor communities to investigate a novel anti—caries agent known as Nano Silver Fluoride (NSF), which has great antimicrobial activity against the main pathogens of the decaying of tooth while being safe towards humans [86]. The anti-caries agent is made from silver NPs, chitosan and fluoride. The study showed a desired effect of its ability to arrest tooth decay. A commercially available dressing, Acticoat, is produced by Smith and Nephew, to fight against infections [87]. Acticoat contains nanocrystalline silver which provides constant release of silver into the wound, creating a barrier to prevent bacterial penetration. The nanocrystalline silver has a broad antibiotic spectrum activity, making it effective against many types of pathogenic strains [87]. Acticoat is also able to inhibit biofilm formation of *P. aeruginosa* and *Acinetobacter baumannii* [88]. Another silver-based NPs is AgTive®. It is a central venous catheters embedded with silver, which can induce bactericidal activity by releasing silver ions into the bloodstream [89]. The rise of the term 'oligodynamic activity' was given to silver due to its biocidal ability against various types of microorganisms [90].

A selenium nanoplatform (Se@PEP-Ru) has excellent fluorescent properties towards bacterial infection due to its high sensitivity which assists in the early

imaging diagnosis. An antibacterial peptide UBI29-41 is linked to the selenium NPs to further enhance the stability of the antimicrobial peptide, allowing specific targeting of bacterial infections and ruthenium complexes have great antibacterial properties while the outer layers are coated with fluorescence [90].

Zhao *et al.* reported a new way to combat against methicillin-resistant S. aureus (MRSA) infection [91]. A probe was designed with the coating of vancomycin-modified polyelectrolyte-cypate complexes on silica NPs (SiO_2-Cy-Van). The probe gets activated by bacteria which causes the dissociation of the polyelectrolyte from the NPs. It is shown that MRSA was able to draw the complex from the NPs onto its own surface, allowing near-infrared fluorescent detection of the MRSA infection. Besides that, the nanoprobes is able to provide a long-term tracking for the development of MRSA infections, giving it the ability to monitor the efficacy of treatments [92].

Taiwan's Cargico Group produced a technology known as Bio-Kill, which is widely used in many various sectors such as hospital, airports and many more. This patented technology uses covalent chemical bonding to attach nano-sized antimicrobials onto a surface, forming a sturdy polymer. The molecules consist of a high-affinity structure with strong electric fields, attracting pathogens and killing it by damaging membrane proteins [93].

A formulated liposomal antibiotic amikacin (Arikace) was designed for the treatment of gram-negative lung infections such as cystic fibrosis which is caused by *P. aeruginosa* [94]. Studies showed when Arikace is inhaled, the liposomes can penetrate the CF sputum and invade the biofilms of *P. aeruginosa*. Additionally, Arikace has a prolonged lung deposition and it is found to reduce infection *in vivo* [94, 95]. Table 2 shows a summary of current types of antimicrobial NPs along with their mode of actions.

8. ANTIBACTERIAL NPS UNDER PRECLINICAL INVESTIGATION

While various materials of NPs are being explored, it is no surprise that some are proceeding to preclinical phase on animal models. Preclinical trials are one step before clinical trials which evaluates its effectiveness, toxicity, and adverse effects to ensure that the drug or substance is safe for human use [98,99]. A study reported in 2019 uses a mixture of copper and silver NPs to understand its bactericidal activity against methicillin-resistant *S. epidermidis* (MRSE). The usage of these NPs was compared with Baneocin, a broad-spectrum antibacterial drug containing a mixture of bacitracin and neomycin. The NPs showed a better effect against MRSE comparing to Baneocin. When treated with Baneocin, the microbial contamination in the wound significantly dropped on day 5 when compared to the start of treatment, and the wound healing effects remained clinically significant till day 10. Whereas, when treated with the NPs, microbial contamination was seen below the clinically significant level on both day 7 and day 10, the wound was found to be sterile with no bacterial infection [100].

Augustine *et al* synthesized silver NPs from *Biophytum sensitivum* plant and incorporated it in calcium peptinate dressing for wound closure application [101]. The 0.5 wt% silver NPs-incorporated calcium pectinate dressing was highly biocompatible to human cells, possessed high cellular uptake and more importantly, showed excellent antibacterial action against *E. coli* and *S. aureus* [101].

In 2018, the same group addressed the same issue using silver NPs-incorporated electrospun polyvinyl alcohol (PVA) membranes [102]. The silver NPs were biologically synthesized using *Mimosa pudica* plant and the NPs could effectively inhibit *S. aureus* and *E. coli.*

Table 2. *Nanomaterial-based systems developed for antimicrobial applications.*

Name	Drug/ molecule	Administration route (model)	Company	Application	Mode of action	References
Silicon 2,3-naphthalocyanine dihydroxide (Nc) and Vancomycin functionalized silica-encapsulated, silver-coated gold NPs (Au@AgNP@SiO_2@Nc-Van)	Silicon 2,3-naphthalocyanine dihydroxide (Nc) and Vancomycin	Sub-cutaneous (female BALB/C mice)	-	Theranostic	• Diagnosis of vancomycin (Van)-resistant enterococci (VRE) through surface-enhanced Raman scattering (SERS). • Antimicrobial properties by photodynamic therapy of VRE	[85]
Selenium nanoplatform (Se@PEP-Ru NPs)	Ruthenium complexes/ polypeptide	Sub-cutaneous and topical (nude BALB/cA-nu mice)	-	Theranostic	• Early diagnosis and antimicrobial property of bacteria by using Ruthenium complex coated on the surface which possess antibacterial and fluorescence properties for imaging of infected locations • Selenium NP are functionalised with antibacterial peptide UBI29-41 (PEP). With the combination of both enhance the stability of the peptide and result in targeted antibacterial therapy	[90]
Silica NPs coated with vancomycin-modified polyelectrolyte-cypate complexes	Vancomycin-modified polyelectrolyte-cypate	Sub-cutaneous (female BALB/C mice)	-	Theranostic	• Near- infrared fluorescence detection of MRSA • Antimicrobial properties by photodynamic therapy of MRSA	[91]
Nano Silver Fluoride	Fluoride	Application on tooth surface (human)	-	Dental caries treatment	• Silver bullet with antimicrobial property	[86]
Bio-Kil®	Inorganic metal and organic quaternary ammonium	Surface decontamination	Carigo Group, Taiwan	Nano-based disinfectant	• Reduce environmental bacteria and have an effect against multidrug resistance organism	[93]
ACTICOAT	Silver nanocrystalline	Topical as a protective covering	Smith+ Nephew	Antimicrobial Barrier Dressings	• Used for wound management. • Effective against most strain of wound pathogens as it has a wider antibiotic spectrum. • Acts as a protecting layer over wound and effective against fibroblasts and keratinocytes	[87]
AgTive	Silver nanoparticles	Intravenous through subclavian or jugular sites (human)	MedeX Medical Inc	Silver-impregnated central venous catheter	• Release silver ions in the bloodstream which has bactericidal activities	[89,97,97]
Arikace	Liposomal amikacin	Inhalation through nebulizer (human)	Insmed Incoporated	Antimicrobial	• Aerosol delivery bactericidal activity	[94, 95]

Moreover, the fabricated membrane was not toxic to red blood cells, fibroblast cells and epithelial cells [102]. On top of that, yiitium oxide [103] and cerium oxide [104] nanoparticles were also explored by the same group to look into wound healing application.

Sosedova *et al.* produced silver NPs encapsulated with poly(1-vinyl-1,2,4-triazole) (PVT) [105]. Antibacterial activity along with the general toxicity, and neurotoxicity were studied in mice and rats respectively. The Ag-PVT NPs was tested against many strains including *E. coli*, *P. aeruginosa* and *K. pneumoniae*. It was found that E. coli was highly sensitive against the NPs as compared to the other strains of bacteria. When tested for toxicity, there were no abnormalities found in the behavior of the animals with E. coli infections. Furthermore, no differences were found between sexes in response to the NPs [105]. Gold NPs coated with chicken egg white (CEW) and 2-mercapto-1-methylimidazole (MMT) was formulated by Lu *et al* [106]. Strains of bacteria including gram-positive, gram-negative, and MDR bacteria were studied. *S. aureus*, *B. cereus*, *E. coli*, *S. enteritidis*, *P. aeruginosa* and MRSA were killed in NPs containing higher concentration of MMT (Table 3). In the *in vivo* study, MRSA were used to infect a 2 cm wound on the model.

The wounds treated with Au@CEW/MMT3 (higher-concentration of MMT) showed scab formation by day 3 along with a 30% reduction of wound size which was not seen on the wounds treated with AU@CEW and cotton gauze [106].

Moxifloxacin, a drug that is commonly used to treat bacterial infections of the respiratory tract, skin, gastrointestinal system and many other organs. Nanocomposite made from chitosan, silver, and sericin loaded with moxifloxacin (CSSM) was produced and it showed wound healing properties and antibacterial effects against *S. aureus*, *S. epidermidis*, MRSA, MRSA 10, MRSA 11, *P. aeruginosa*, and *A. baumannii* in Male Sprague Dawley Rats. CSSM was used to treat burn-injured model and was found to possess quicker wound healing and by studying the histological measurement, it showed well-formed structures (formation of blood vessels, collagen deposition, and epidermal regeneration). All strains that were tested against the nanocomposite had excellent bactericidal activity except against MRSA [107].

Aghamoosa *et al.* studied the synergism of silver and curcumin to treat burned wounds [108]. Silver-curcumin NPs are formulated in this study and is tested on both bactericidal and its toxicity effect. Four strains of *Salmonella typhymurium* were used and it was found that the NP was able to reduce the mutation rates in all tested strains in a dose-dependent manner (Table 3). Using silver-curcumin NPs as dressing for burn wound in Adult Male Wistar Rats and rabbits, a significant improvement was observed as compared to the control. The acute skin irritation and corrosion study using the NPs had no sign of toxicity in the first 24 hours but a slight erythema was seen after, but soon disappeared before the 72 hour [108].

Silver NPs was found to inhibit the metabolic activity of a lung cancer cell line A549, and no connection was found between the cytotoxicity and the size of NP. When it was injected into the mice model, the distribution of the NP was mostly found in the liver, spleen, and lungs. The effect of the NPs was time and dose-dependent and the high bactericidal activity in A549 cell line was seen when was treated with the smallest NPs [109].

Table 3. *Metal NPs in preclinical trials/undergoing in vivo testing.*

Name	Metal(s)	Bacteria		Effect(s)	Model	Administration route	Reference
		Gram positive	Gram negative				
Copper and silver NPs	Copper, silver	Methicillin-resistant S. *epidermidis*	-	Antibacterial	White out-bred rats	Topical on skin wound	[100]
Ag(0) particles encapsulated with poly(1-vinyl-1,2,4-triazole) (PVT)	Silver	S. *aureus*, *E. faecalis*	*E. coli*, *P. aeruginosa*, *K. pneumonia*	Antibacterial, toxicity	Male white rats and white mice	Intragastric injection	[105]
Gold NPs coated with chicken egg white (CEW) and 2-mercapto-1-methylimidazole (MMT)	Gold	S. *aureus*, *B. cereus*, MRSA	*E. coli*, *S. enteritidis*, *P. aeruginosa*	Antibacterial, wound healing	New Zealand rabbits	Topical on skin wound	[106]
Triple-component T nanocomposite (chitosan-silver-sericin) films loaded with moxifloxacin	Silver	S. *aureus*, S. *epidermidis*, MRSA, MRSA 10, MRSA 11	*P. aeruginosa*, *A baumannii*	Antibacterial, wound healing	Male Sprague Dawley rats	Topical on burn wound	[107]
Silver-curcumin nanogel	Silver	-	*S. typhimurium strains TA100, IA98, YG1029, YG1021*	Antibacterial, Toxicity	Adult male Wistar rats, female rabbits	Topical on skin (rats) and burn wound (rabbits)	[108]
Silver NPs	Silver	-	*E. coli*	Antibacterial, toxicity	Male C57BI/6 mice	Intraperitoneal injection	[109]
TaON-Ag	Silver	S. *aureus*, coagulase-negative Staphylococcus	*E. coli*, *P. aeruginosa*	Antibacterial, wound healing	Rat osteomyelitis model	Intrafemoral injection	[110]
Phyto-engineered gold NPs (AuNPs)	Gold	S. *epidermidis*	*E. coli*	Antibacterial	BALB/c mice	Topical on skin wound	[111]

TaON-AG coated titanium NPs showed great antibacterial effect on MSSA, MRSA, *P. aeruginosa*, and *E. coli*. When compared to the control, *E. coli* was also found to be distorted and cytoplasmic membrane wrinkles were found which is believed to have led the leakage of the cytoplasm and cell lysis. When it was observed during the *in vivo* osteogenic assay, no side effects were seen during the healing process of the fracture. The TaON-Ag-coated Ti needles were placed to facilitate the fracture was found to be infected with *S. aureus* or *E. coli* until the 14 day [110].

A phyto-engineered gold nanoparticle in the *Acalypha indica* leaf extract was formed and it had very strong zone of inhibition of bacterial growth against *S. epidermidis* and *E. coli* which is 31 mm and 26 mm diameter, respectively. The extract contacting the NPs showed better re-epithelization and higher healing process in BALB/c mice when compared to its control. This showed that the extract containing NPs speed up the healing process [111].

As mentioned above, one of the advantages of using nanoparticles was its ability to be used as nanocarrier. Various nanoparticles or nanomaterials loaded with

anticancer drugs are currently being used for cancer patient treatment such as Onivyde and Vyxeos (Table 1). Similarly, when antibiotics are loaded onto nanoparticles, synergistic antibacterial effect may lead to maximum killing of target bacteria. For example, the synergistic action of novel cetyltrimethylammonium bromide (CTAB) with silica-nanoparticles (MPSi-CTAB) resulted in effective killing against Methicillin-resistant *Staphylococcus aureus* and inhibited biofilm of *Staphylococcus epidermidis* [112]. In another report, rhamnolipid (RHL)-coated Fe_3O_4 nanoparticles (NPs) with p-coumaric acid (p-CoA) and gallic acid (GA) antimicrobial drugs significantly interfered the growth of drug-resistant *S. aureus* (MRSA & VRSA) and virulent *E. coli* (O157:H7, O26:H11, and O78:H10) as well as inhibited more than 50% of the biofilm [113]. To date, Arikayce is the only FDA-approved nanomaterial-based drug (Table 1) used against mycobacteria avium complex-caused lung disease while many are still under investigation.

In other studies, nanoparticles can also be used directly (without drug loading or conjugation) in combination with antibiotics for antimicrobial treatment. For example, silver NPs in combination with polymixin B and rifampicin could synergistically kill carbapenem-resistant strain of *Acinetobacter baumannii* both *in vitro* and in *in vivo* infected mouse [114]. Similarly, Krychowiak *et al* reported that silver NPs could synergistically kill drug-resistant clinical isolates of *S. aureus* when combined with naphthoquinones [115]. More recently, chitosan-copper and cobalt ion nanoparticles showed excellent antibacterial action against MRSA and antibiotics-resistant *E. coli* when combined with cefepime or penicillin [116]. The same group also showed the combination treatment led to the rupture of cell membrane and the silencing of gene expression vital to the bacterial growth.

Table 4. *Metal NPs in various phases of clinical trials.*

Name	Metal	Uses	Administration route	Phase	Status	Institution	ClinicalTrials.gov Identifier	Reference
Silver NPs	Silver	Tinea pedis, Capitis and Versicolor infection	Topical gel	1	Unknown	Al-Azhar University	NCT03752424	[117]
Copper or zinc NPs	Copper or zinc	Dental caries	Dental adhesive	Non-applicable	Unknown	University of Chile	NCT03635138	[118]
Chitosan and/or titanium NPs	Titanium dioxide	Pulpitis, dental caries	Filling on cavity over caries	3	Completed	British University (Egypt)	NCT04365270	[119,120]

9. ANTIBACTERIAL NPS UNDER CLINICAL TRIALS

Being an expanding field, there are numerous NPs under clinical investigation (Table 4), for example, a trial that first started in 2018, focused on the use of silver NPs to treat for foot fungal infection. The NPs were prepared in a cream formulation and the cream was tested primarily for its antimicrobial effect for six months at different dosages. The secondary outcome taken into consideration is the stability of the product and its shelf life for three months [117]. For the treatment of dental caries, it is believed that with the addition of metal components such as copper/ zinc NPs to dental adhesive would provide antimicrobial effect along with its resistance from enzyme degradation. This study measured the colony forming units (CFU) of *Streptococcus mutans* and the microtensile bond strength [118]. Another study that focuses on the treatment of dental caries has recently completed its phase 3 clinical trials [119]. The glass-ionomer cement was modified with

chitosan and titanium-dioxide nano-powder and results showed significant improvements in the antibacterial properties [120].

10. KEY CHALLENGES IN DEVELOPMENT OF FDA-APPROVED ANTIBACTERIAL NPS

A typical process of getting drug approval from the FDA requires many data and requires many stages starting from having an idea to it being a marketed product, as depicted in **Figure 2** [124, 125]. Most drugs and medical devices are required to be FDA approved before making it available to the public [123]. Drug manufacturers may undergo two types of procedures to market their drugs, either by fulfilling the over-the-counter (OTC) monograph or the full procedure, where the manufacturers are required to submit lab, animal, and clinical test data to the Center for Drug Evaluation and Research (CDER) to determine the effectiveness and safety of the drug. In the event the drug fulfills the OTC monograph,

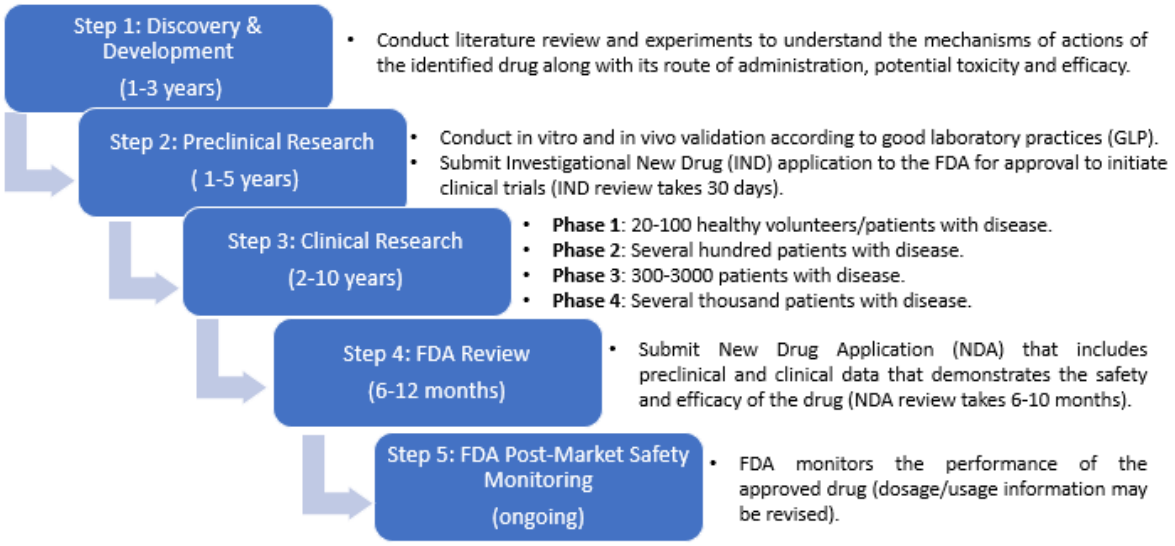

Figure 2. *The overall process of getting FDA approval for investigational drugs.*

FDA approval will not be needed for the marketing of the drug [123].

Other than drugs, the FDA also approves medical devices that are categorized depending on their risks to the patient and user. The higher the classification (Class III), the higher the risks. In the lower risks categories (Class I & III), a premarket notification known as 510(k) is required to be submitted to FDA. This will demonstrate if the submitted device is substantially equivalent to a currently available device [124,125,126].

According to a guidance document released by the FDA in 2014 [127], there are certain screening tools that may be applied to all FDA-regulated NPs materials/ products. Points such as whether the materials/ product consist of more than one
dimension or have internal or surface structure in the nanoscale range and whether the materials/ product is engineered to exhibit specific properties (physical, chemical or biological effects) as long as the size is up to one micrometer. The regulation of nanotechnology by the FDA is dependent on the characteristics and its biological effects of the product and its intended usage. As abovementioned, Acticoat Surgical Dressing, the product required a submission of the 510(k) premarket notification as it is considered as an unclassified medical device, meaning it is lower than a class I medical device [128].

Products that have better efficacy than the available drugs or are treating conditions that do not have proper treatments may be released to market earlier. This can be seen in the previously mentioned product, Arikace. Arikayce was approved under the accelerated approval and the LPAD as it targets the rare *Mycobacterium avium* complex (MAC) [129].

The Accelerated Approval Program allows for an earlier approval for drugs that treat serious medical conditions and achieves some medical needs based upon a surrogate endpoint. Nonetheless, manufactures are required to conduct phase 4 confirmatory trials to understand the expected clinical benefits. If the trial shows positive clinical benefits, the FDA will be able to grant their approval [130]. The drug can also be approved through the Limited Population Pathway for Antibacterial and Antifungal Drugs (LPAD pathway) – this pathway assists the approval of antibacterial or antifungal drugs for the treatment of rare infections.

Nanotechnology is still a relatively expanding field; therefore, its safety and toxicity needs to be evaluated before they can be mass produced and commercialized. Studies has shown that metal or metal oxide NPs, although inert in their bulk counterpart, have different activities and properties and therefore, their biological properties and toxicity both in vitro and in vivo was evaluated. The solubility of NPs is not really perfect and slightly unstable. However, the dissolved or eluted metals and metal ions have certain toxicity effect on the body. The NPs may have non-specific interactions when they come into contact with biological constituents and disrupt cellular processes, leading to various unexpected adverse effects. For instance, the metal moieties may interact with biomolecule intracellularly which results in oxidative stress followed by apoptosis and inflammation of the cells. Normally, cells have mechanisms that regulates oxidative stress; however, the metal moieties can cause continuous, repetitive and long-term oxidative stress and inflammation which will results in fibrosis and cancer. Another possibility is that the NPs may trigger the immune system or cause allergies in certain individuals. Nevertheless, NPs with proper coating and surface modification could enhance the stability and mitigate the toxicity effect [131,132].

The optimal dose of NPs for clinical use is extremely important, and perhaps this is the biggest challenge of all. Formation of biodegradable NPs can increase the therapeutic efficacy while minimizing side effects. For examples, liposomes and other polymeric NPs have been studied to be used for drug delivery as they are less toxic and could protect drugs from degradation. For this purpose, inorganic NPs are preferred over organic NPs which are relatively toxic to the biological systems [133].

Nanotechnology has a lot of potential in the medical and research field. Study has been conducted on the diagnosis effect and the treatment potential of the metal and metal oxide NP. However, studies are conducted to investigate the potential theragnostic ability of the metal and metal oxide NP. One of the potential metal oxide NPs that have theragnostic ability is the superparamagnetic iron oxide due to their unique magnetic properties. The magnetic ability gives Iron oxide NPs the ability to target tumor efficiently which is favorable for theragnostic ability. For the diagnosis of cancer, the superparamagnetic iron oxide NPs (SPIONs) improve the contrast of magnetic resonance imaging and the SPIONs' can be modified with tumor specific receptor which will improve their effectiveness in detecting and exert their activity in the tumor milieu [134]. Silver and gold NPs also have the potential to act as theragnostic. They possess versatile properties such as physical and chemical properties which arise from the shape and size of the NPs and their unique therapeutic, electric, catalytic and photonic

properties as noble metal NPs make them potential subject for theragnostic activities [135].

Metal and metal oxide NPs possess many desirable characteristics which boost their production industrially. Gold and silver NP are manufactured widely in the market in different forms such as silicon coated, sphere shape or organic gold or silver. In the market, organic gold NPs can amount to €20 to €40 per 1 milligram. PVP stabilized gold NPs are relatively costlier compared to the organic gold NPs as the market price is €160 for 1 milligram. Furthermore, the more functionalized the gold NPs are, their price rises and can reach up to €200. Silver NPs pricing is similar to that of gold NP; however, platinum NPs are more expensive in the market where the price can amount to €1900 per gram. Metal oxide NPs are not as costly as metal NPs due to their manufacturing procedure being hydrothermal technique which is advantageous as speed, quality and yield, low temperature and operational ease reduces the cost of manufacture and subsequently their market price [136].

Therefore, the process of getting an FDA approval is dependent on the materials or products that are released to the market. The procedure varies and it follows the intended use of the product itself. The effectiveness and safety of the product is thoroughly evaluated through information provided by the researchers and it is compared to the current needs of the population and the effectiveness of it when comparing to publicly available products.

11. CONCLUSION

Within the last decade, researches incorporating nanotechnology has been surfacing involving the different approaches nanotechnology that can be used in the biomedical field. Metal and metal oxide NPs have shown potential antibacterial properties which is very favorable in a world where multi-drug resistant bacteria are becoming more common. Taking into account the benefits that metal and metal oxide NPs provide due to their antimicrobial properties, strategies to develop and utilize them as treatments against bacterial infections are on the rise. In this review, we discussed the studies related to the antimicrobial effect of the metal and metal oxide NPs as well as the mechanism of action involved in their antibacterial activities. We also introduced several metal and metal oxide NPs that have already reached the market, NPs that are undergoing pre-clinical trials and those that are currently under clinical trials. While there are countless NPs under investigation, only a few reached the phase of clinical trials and even fewer gets approved to be released in the market. Therefore, to ensure these pre-clinical studies are translated into clinical practice, future research must be undertaken to further understand the mechanisms of actions of antibacterials NPs and their possible interactions with various components within the human body in order to ascertain the potential side effects and long-term safety of NP-based antibacterial therapy, especially on the risk of accumulation in the liver and kidneys. This can be done by developing *in vitro* and *in vivo* models capable of recapitulating the biological environment to validate the safety and efficacy of this therapy before the commencement of clinical trials. With this, the transition of laboratory research to clinical applications can be improved, ensuring timely access to patients in need of this promising treatment.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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