Environmental Science and Pollution Research (2021) 28:26252–26268
https://doi.org/10.1007/s11356-021-13373-z

REVIEW ARTICLE

Recent development in antimicrobial activity of biopolymer-inorganic nanoparticle composites with water disinfection potential: a comprehensive review

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Received: 7 July 2020 / Accepted: 5 March 2021 / Published online: 31 March 2021

Abstract

Nowadays, water-borne diseases including hepatitis remain the critical health challenge due to the inadequate supply of potable and safe water for human activities. The major cause is that the pathogenic microorganisms causing diseases have developed resistance against common techniques used by sewage water treatment plants for water disinfection. Therefore, there is a need to improve these conventional water treatment techniques by taking into consideration the application of nanotechnology for wastewater purification. The main aim of this paper is to provide a review on the synthesis of biopolymer-inorganic nanoparticle composites (BINCs), their used as antimicrobial compounds for water disinfection, as well as to elaborate on their antimicrobial mechanism of action. The microbial properties affecting the activity of antimicrobial compounds are also evaluated.

Keywords Water disinfection · Biopolymer-inorganic nanoparticle composite · Antimicrobial mechanism · Antimicrobial susceptibility test and nanotechnology

Introduction

Nanotechnology is currently regarded as the most effective and promising technique to overcome issue related to long-term water treatment (Alonso et al. 2011; Adeleye et al. 2016; Vunain et al. 2016; Momba et al. 2017). The application of nanotechnology in water treatment involves the use of nanomaterials (NMs) acting as filters, adsorbents, reactive agents, or disinfectants which enhance water treatment and recycling (Alonso et al. 2011; Qu et al. 2013; Bora and Dutta 2014; Adeleye et al. 2016; Vunain et al. 2016; Momba et al. 2017).

In this regards, metal oxide and metal nanoparticles (NPs) such as titanium dioxide (TiO_2), zinc oxide (ZnO), silver (Ag), gold (Au), and copper (Cu) have been mostly used as antimicrobial compounds (AMCs) or antimicrobial agents in water treatment as well as in other applications (Boomi and Prabu 2013; Tran et al. 2013; Stankic et al. 2016; Wang et al. 2017). However, in recent studies, cases of bacterial resistance against the antimicrobial agents and formation of biofilm have been reported (Domènech et al. 2013; Beyth et al. 2015; Palza 2015). In order to overcome this issue, the development of polymeric nanocomposites have attracted a lot of attention (Boomi and Prabu 2013; Zhang et al. 2013). Polymeric nanocomposites can be defined as hybrid nanomaterials containing inorganic nanoparticles embedded into a polymer matrix. They are macromolecular nanomaterials and possess extraordinary multifunctional properties such as electrical-dielectric, optical, thermal stability, magnetic properties, antimicrobial, and mechanical properties (Paul and Robeson 2008; Palza 2015). Additionally, inorganic nanoparticles incorporated into the polymer matrix helps to control the shape, size, and structural morphology of the nanoparticles (Domènech et al. 2013; Zhang et al. 2013).

Responsible editor: Angeles Blanco

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Nowadays, polymer-inorganic NPs composites have been considered as emerging nanomaterials used in wastewater disinfection or in other applications to enhance the antimicrobial activity of inorganic (metal or metal oxide) NPs. This is because the polymer nanocomposite materials are non-volatile, stable, and perfectly bind to the interested surface (Beyth et al. 2015). They also exhibit strong electronic interaction between the polymer matrix and metal NPs (Boomi and Prabu 2013) and have demonstrated strong antimicrobial activity (Kenawy et al. 2007; Beyth et al. 2015; Kamaruzzaman et al. 2019). Moreover, among the polymer nanocomposites, biopolymer nanocomposites are mostly preferred because biopolymer matrices such as cellulose, chitosan, alginic acid, agarose, pectin, and starch including ß-cyclodextrin polymers are environmentally green in nature. In addition, they are biodegradable, readily available, easy to scale up, and not expensive (Datta et al. 2008; Dhanavel et al. 2018; Sivaranjana et al. 2018). These biopolymer matrices, due to their oxygen-rich functional groups and great biocompatibility, also act as good reducing and stabilizing or capping agent for nanoparticles. Furthermore, they help to prevent the aggregation of NPs during the synthesis process (Datta et al. 2008; Zhang et al. 2013; Dhanavel et al. 2018).

Research work on BINCs, that is, their application and performance as antimicrobial compounds for wastewater disinfection, is still scarce. Hence, the objective of this article is to give a review of the different synthesis methods of BINCs and their application as antimicrobial compounds and to elaborate on antimicrobial susceptibility testing methods appropriate for an efficient assessment of the antimicrobial activity of nanomaterials (metal nanoparticles and polymer-metal nanoparticle composites). Furthermore, the antimicrobial mechanisms and the microbial properties affecting the activity of antimicrobial compounds are discussed. The performance of antimicrobial compounds in real wastewater solutions is also examined.

Overview on the synthesis of biopolymer-inorganic nanoparticle composites (BINCs)

The development of biopolymer-inorganic (metal or metal oxide) nanoparticle composites offers green and novel approaches to overcome limitations related to environmental remediation (e.g., water treatment), medical, electronic, and renewable energy applications (Datta et al. 2008; Zheng et al. 2015; Dhanavel et al. 2018). This is because of their multifunctional properties which arise from the combined properties of material components present in the BINCs (Paul and Robeson 2008; Palza 2015; Palza et al. 2015). These biopolymer nanocomposites can be synthesized using a variety of methods such as the in situ (Vigneshwaran et al. 2006; Trandafilovic et al. 2012; Domènech et al. 2013; Palza 2015; Singh and Ambika 2018), ex situ (Domènech et al. 2013; Palza 2015), inter matrix synthetic (or intercalation) method, film casting-dip coating-physical mixing (Vigneshwaran et al. 2006; Travan et al. 2011; Wang et al. 2012a), as well as direct mixing by either melt compounding or solution mixing (Pinto et al. 2009; Singh and Ambika 2018).

Ex situ and in situ methods

Figure 1 illustrates the difference between the ex situ and in situ method used for the synthesis of polymer-metal nanoparticle composites. The ex situ method involves first the preparation of the NPs, followed by their addition to the polymer matrix used as a dispersion medium (Campelo et al. 2009; Triebel et al. 2011; Palza 2015; Leudjo Taka et al. 2017). On the other hand, the in situ method can be defined as the direct production of the inorganic metal or metal oxide NPs in the presence of polymer matrix in the medium. In this way, the polymer will act as a stabilizer or capping agent to prevent the agglomeration of NPs and to control the NPs size and shape during the preparation. In addition, the in situ method is widely used and includes the chemical processes such as precipitation (Sambhy et al. 2006), reduction (Kemp et al. 2009a, b; Travan et al. 2011; Dhanavel et al. 2018), sol-gel (Singh and Ambika 2018), oxidation, and electrospinning methods (Zheng et al. 2015; Nthunya et al. 2017). Figure 2 illustrates an example of the synthesis of chitosan/palladium NPs biopolymer nanocomposite obtained by in situ chemical reduction method (Dhanavel et al. 2018).

The quality of polymer-metal nanoparticle composites prepared by the in situ method has been reported to be affected by some factors which might reduce the purity and homogeneity of the polymer nanocomposites (Zhang et al. 2013). Some of these factors are the inability to eliminate the excess of ions added during the reduction process and the different experimental conditions which may be necessary for the dressing and reducing agent. In order to overcome these limitations, a one-step in situ method has been developed whereby a biopolymer (e.g., chitosan) with both stabilizing and reducing ability is used (Wang et al. 2010; Zhang et al. 2013). Therefore, the addition of a reducing agent (e.g., sodium borohydride ($NaBH_4$), ascorbic acid, hydrogen gas (H_2)) into the reaction media is no more critical, and this has resulted to highly pure polymer-metal NPs composites with homogeneous size and shape of metal NPs (Zhang et al. 2013).

Inter matrix synthetic (or intercalation) method

This method can be described as a synthetic host-guest method which involves the ion exchange properties of

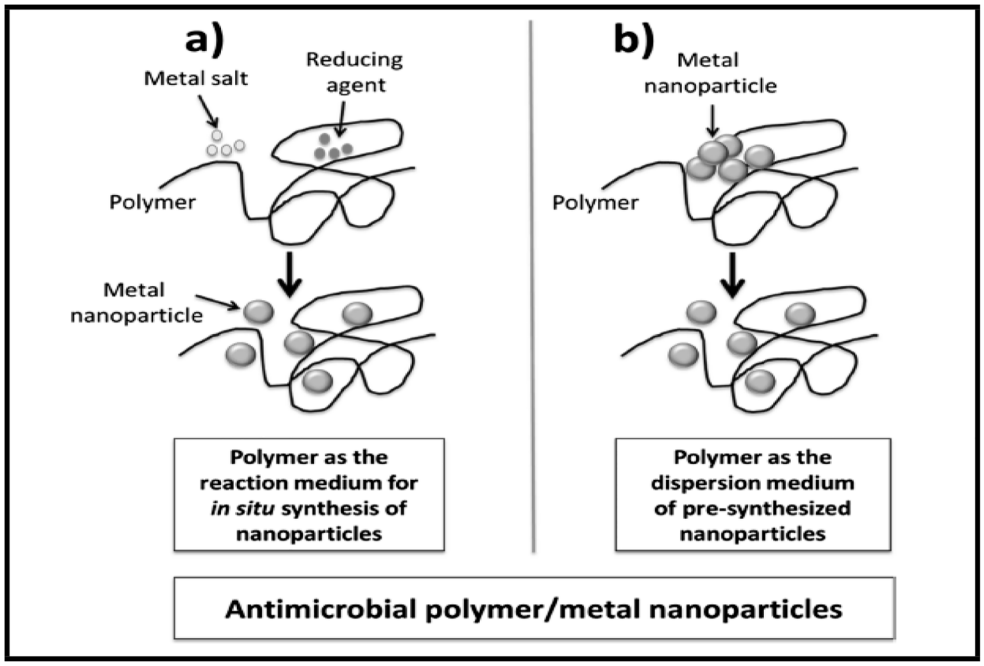

Fig. 1 Illustration for the preparation of an antimicrobial polymer/metal NPs composites: (**a**) synthesis by the in situ method and (**b**) synthesis by ex situ method (Palza 2015)

some polymeric matrices (Fig. 3) (Domènech et al. 2013). The binding of ionic precursors initiates it to the functional groups present in the polymer matrix (Domènech et al. 2013; Singh and Ambika 2018). The metal ions are then attached on the polymer matrix followed by in situ chemical reaction (e.g., precipitation, reduction, or oxidation) to produce a polymer-metal NPs composite with well-dispersed small NPs embedded in the polymer matrix (Domènech et al. 2013; Singh and Ambika 2018).

Additionally, the challenges in preparing nanocomposites include control of their synthesis through the preparation method used, ensuring the compatibility of their different material components and special properties (Zheng et al. 2015).

The application of BINCs for wastewater disinfection processes based on antimicrobial susceptibility testing methods

Antimicrobial susceptibility testing methods

Antimicrobial susceptibility testing methods are generally necessary for guiding the treatment of the different types of microbial infections (Varaldo 2002; Pulido et al. 2013). In the context of water purification, these antimicrobial susceptibility testing methods are advantageous because they help in knowing the antimicrobial activity of the nanomaterials (NMs) to be used as disinfectants for water treatment. Then, once knowing the antimicrobial activity of these NMs, it will allow the safe use of these nanomaterials as disinfectants by

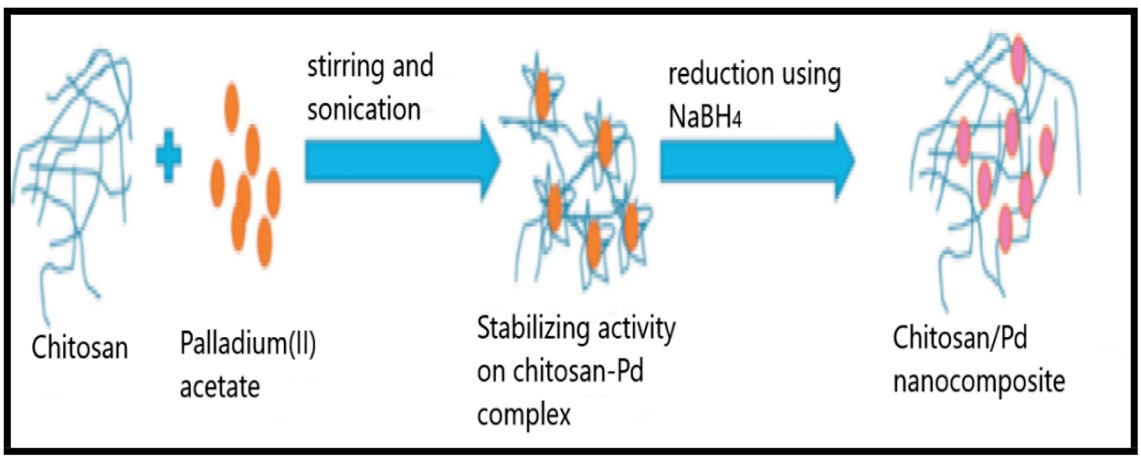

Fig. 2 Illustration of the in situ reduction method for preparation of chitosan/palladium NPs biopolymer nanocomposite (Dhanavel et al. 2018)

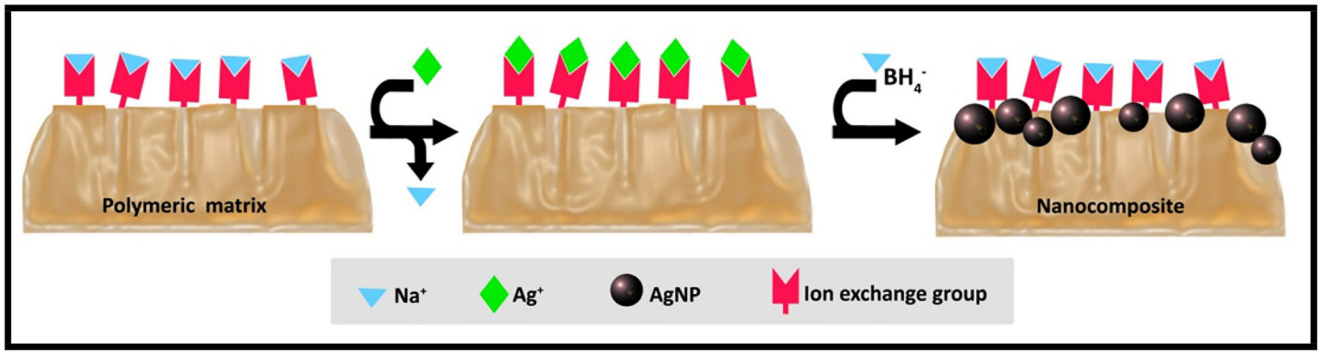

Fig. 3 A pictorial description of the synthesis of polymer-inorganic NPs composite by inter matrix synthetic method (Domènech et al. 2013)

incorporating them into storage tanks and distribution pipe surfaces in order to avoid biofilm formation, microbial pollution, and deterioration (Berekaa 2016).

These antimicrobial susceptibility testing methods can be divided into two categories. The first employed methods which evaluate the antimicrobial activity of the tested compound (e.g., drug or nanomaterial) by measuring bacterial growth in the presence of the compound (e.g., nanomaterial) being tested. The second employed molecular techniques or protocols which have been designed for the rapid detection of antimicrobial compound's effect. The widely used methods in the first category are the agar disc diffusion (Fig. 4), agar dilution (Fig. 5), and broth dilution (macro- and microdilution) (Fig. 6). These methods are highly standardized and sensitive, not complicated, and easy to operate. For instance, Bachir et al. (2017) have synthesized β-cyclodextrin nanosponge (CD NS) by polycondensation method using naphthalene dicarboxylic acid as a cross-linking agent and shown the antimicrobial activity of their synthesized CD NS against Salvia officinalis essential oil, *Microsporum canis*, and *Candida albicans* by agar dilution method for the determination of minimum inhibitory concentrations and minimal fungicidal concentrations (Bachir et al. 2017). Hodyna and co-workers have developed 1-dodecyl-3-methylimidazolium tetrafluoroborate ionic liquid/β-CD complex and demonstrated its antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Candida albicans* by standard disc diffusion method (Hodyna et al. 2016).

Additionally, there are only very few research studies on the antimicrobial activity of BINCs using these highly standardized susceptibility test methods. Anitha et al. (2012) have narrated on the preparation of cellulose acetate/ZnO NPs biopolymer nanocomposites using the electrospinning method to obtain fibrous membranes. The inhibition effect of the prepared fibrous membrane biopolymer/ZnO NPs nanocomposite was evaluated by the disc diffusion method against Gram-positive and Gram-negative bacteria strains. From the results obtained, the inhibition effect was the greatest against *Staphylococcus Aureus* (Anitha et al. 2012). Bober and co-workers have synthesized biopolymer cellulose/polypyrrole/Ag NPs composite by in situ one-step chemical polymerization method. The nanofibrillated biopolymer nanocomposite obtained has demonstrated intense antibacterial activity

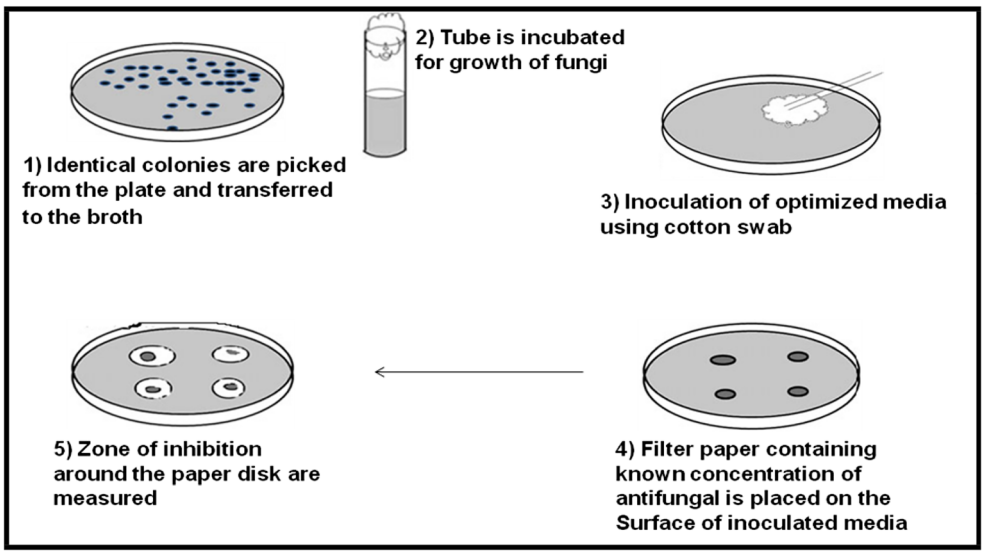

Fig. 4 Illustration of the agar disc diffusion method: this is an example of standard protocol for antifungal tests (Thatai and Sapra 2016)

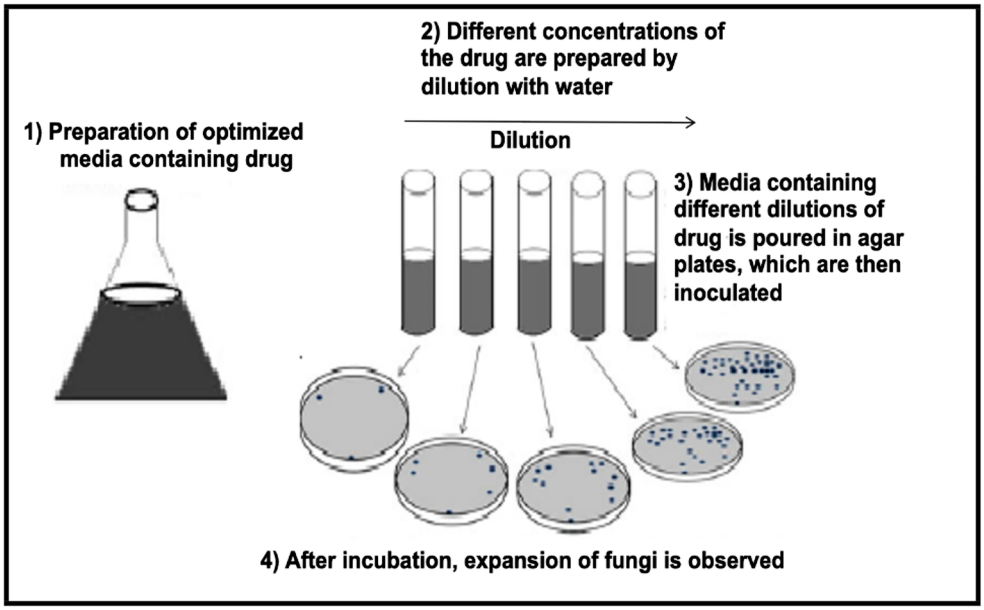

Fig. 5 Illustration of the agar dilution method: the drug is the antimicrobial compound; this is an example of standard protocol for antifungal susceptibility test (Thatai and Sapra 2016)

against *Staphylococcus Aureus*, a Gram-positive bacteria subjected to susceptibility test by agar disc diffusion (Bober et al. 2014).

Said–Galiev and co-workers have reported on the preparation of Ag and Cu NPs/chitosan biopolymer nanocomposites. The synthesis was achieved by incorporating the metal ions into the biopolymer chitosan present in supercritical carbon dioxide medium and then followed by reduction under hydrogen to obtain metal NPs/chitosan biopolymer nanocomposites (Said-Galiev et al. 2011). The antibacterial activity of the synthesized biopolymer-metal NPs composites was investigated by the agar dilution method, followed by the evaluation of colony-forming units (CFU). Cu NPs/chitosan biopolymer nanocomposite has shown bacteriostatic activity against the tested bacterial strains, while Ag NPs/chitosan biopolymer nanocomposite has exhibited bactericidal activity (Said-Galiev et al. 2011). A bactericidal antimicrobial agent is known to kill the bacteria, whereas an antimicrobial agent which is slowing down the growth of the microbe is considered as bacteriostatic (Owuama 2017).

Davoodbasha et al. (2015) have conducted the synthesis of cellulose/silver polymer nanobiocomposites using discharging plasma into a mixture solution of cellulose and silver nitrate, followed by lyophilization and cross-linked process with UV irradiation. The polymer-metal NPs nanobiocomposites obtained were used as an antimicrobial agent against bacteria and fungi. The antimicrobial activities were investigated by both disc diffusion and macrodilution methods. The cellulose/silver polymer nanobiocomposites synthesized demonstrated a bactericidal activity toward Gram-negative bacteria, whereas they did not effectively inhibit the growth of fungi (Davoodbasha et al. 2015).

Nthunya and co-workers have synthesized ß-cyclodextrin/cellulose acetate/silver and silver-iron NPs biopolymer nanocomposites using the in situ electrospinning method followed by UV-photochemical reduction for the reduction of metal ions (Ag^{+} and Fe^{3+}) to their zerovalent state (Nthunya et al. 2017). The biopolymer-metal NPs composites obtained were tested as wastewater disinfectants. After evaluation of the antimicrobial test by microdilution method, the synthesized biopolymer-metal NPs composites demonstrated strong biocidal effect against the tested bacteria strains (Nthunya et al. 2017).

Dhanavel and co-workers reported on the preparation of chitosan/palladium NPs biopolymer nanocomposites by chemical reduction method and used the prepared biopolymer-metal NPs composites as an antimicrobial agent against Gram-negative and Gram-positive bacteria. Lower values of minimal inhibition concentrations (MIC) were obtained by microdilution method against Gram-negative *Proteus sp.* and Gram-positive *Staphylococcus aureus* as well as *Bacillus cereus* disc. Disc diffusion method was also used to investigate the antimicrobial activity of the synthesized chitosan/palladium NPs biopolymer nanocomposites (Dhanavel et al. 2018).

Furthermore, these widely used antimicrobial susceptibility test methods cited above are mostly recommended by the international standard guideline protocols such as CLSI (Clinical and Laboratory Standards Institute) (Clinical and Laboratory Standards Institute 2012a, 2012b) and EUCAST (the European Committee on Antimicrobial Susceptibility Testing) (Eucast 2010). However, Kourmouli et al. (2018) and Leudjo Taka et al. (2020) have revealed that the antimicrobial susceptibility test results obtained based on the standard disc diffusion method are not reliable for the

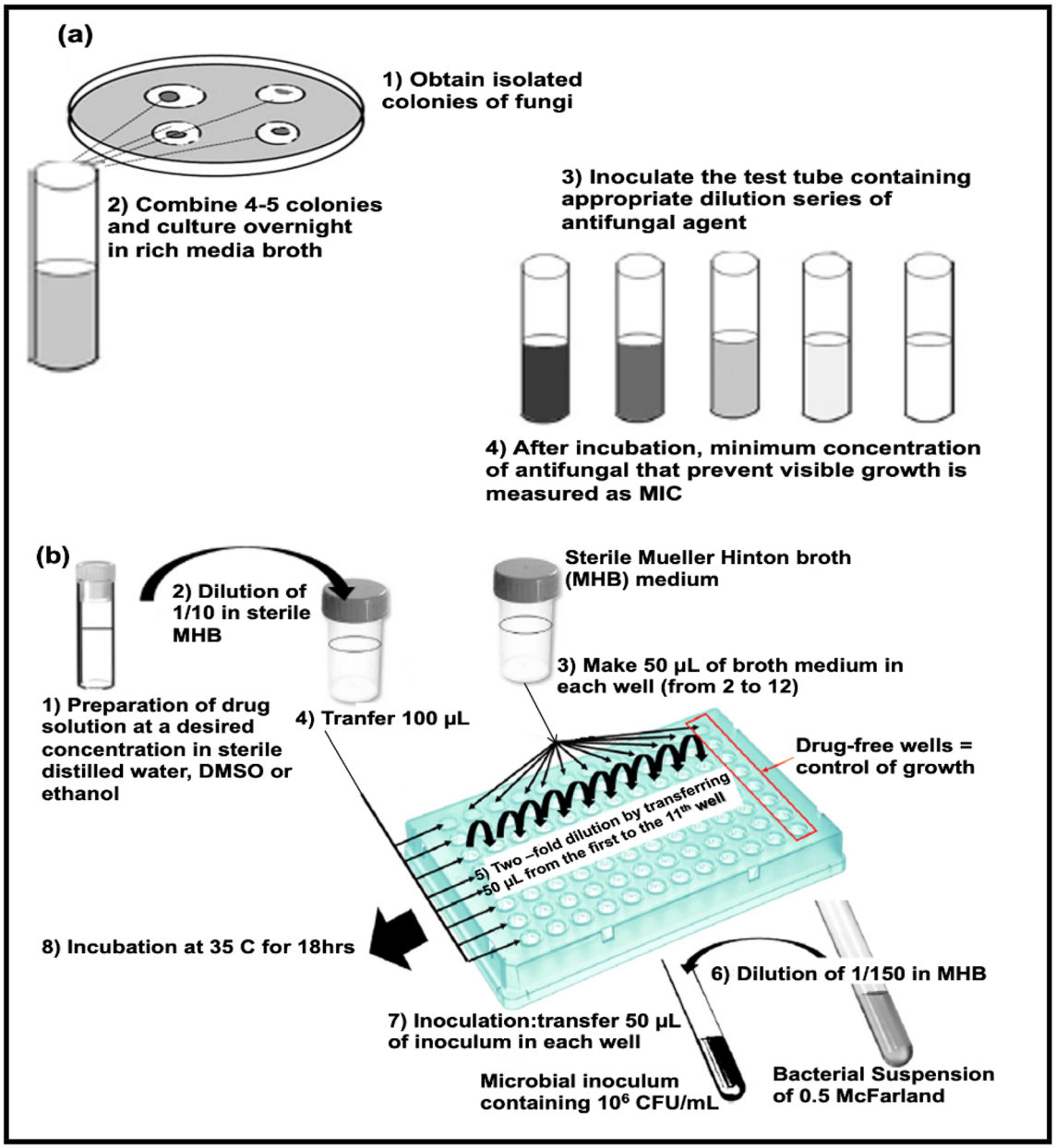

Fig. 6 Illustration of the broth dilution method for antimicrobial susceptibility test: (**a**) macrodilution (e.g., antifungal test) and (**b**) microdilution (e.g. antibacterial test) (Balouiri et al. 2016; Thatai and Sapra 2016)

antimicrobial activity evaluation of nanomaterials (e.g., metal NPs and polymer-metal NPs composites) due to their low diffusivity. In their studies, these researchers have demonstrated that nanomaterials have poor solubility in dimethyl sulphoxide (DMSO) (a solvent commonly used to dissolve the AMC for antimicrobial susceptibility tests). This poor solubility in DMSO has been shown to prevent AMC from penetrating through the pore of the culture media (Kourmouli et al. 2018; Leudjo Taka et al. 2020).

Additionally, other methods of the first category such as the bioluminescence assay, time-kill test, thin-layer chromatography (TLC)-bioautography, cytofluorometric flow method, and the commercial automated system (the Vitek systems from bioMerieux, the Microscan WalkAway system from Siemens, and the phoenix-automated microbiology system from BD diagnostics), are not commonly used because of their complexity (Kourmouli et al. 2018; Pulido et al. 2013). These methods also require specified equipment and more evaluation for standardization and reproducibility. However, the advantage is that they can give rapid results of the tested compounds' antimicrobial activity and a better understanding of the effect of these tested compounds on the viability and cell damage imposed on the tested microbes.

The second category of antimicrobial susceptibility test employed molecular techniques or protocols such as polymerase chain reaction (PCR)-based technique, cell lysis-based approaches, microarrays, whole-genome sequencing, matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOFMS), and microfluidics (Brook et al. 2013; Pulido et al. 2013) which have been developed for rapid detection of the antimicrobial compound's effect. However, these techniques are not recognized as highly standardized protocols, and they require the use of complicated and specific equipment, which are costly (Pulido et al. 2013). Table 1 summarizes the various techniques which have been used to assess the susceptibility of microorganisms in various studies.

Factors affecting the antimicrobial susceptibility testing

The factors, such as the media growth type, the inoculum preparation method, inoculum size, the endpoint determination, the incubation time, and temperature, have been found to affect the antimicrobial susceptibility testing methods (Balouiri et al. 2016; Thatai and Sapra 2016).

Type of growth media

The type of growth medium should be chosen based on the microorganism to be tested. Its composition should be chemically defined in such a way that it can support the growth of the microorganism and should not interfere with the antimicrobial agent (tested compound) (Balouiri et al. 2016; Thatai and Sapra 2016). According to the international standard guideline protocols CLSI (Clinical and Laboratory Standards Institute 2012a, b) and EUCAST (Eucast 2010), the media mostly recommended are the nutrient agar and nutrient broth media for bacteria susceptibility testing, whereas for fungi susceptibility testing, RPMI 1640, sabouraud agar, and potato dextrose agar media are appropriate to use (Balouiri et al. 2016; Thatai and Sapra 2016).

Inoculum preparation and size

The minimal inhibition concentrations (MICs) of the antimicrobial compounds (AMCs) have been shown to increase with increasing inoculum size (Misra et al. 2011). It is recommended by the CLSI or EUCAST that the size of the inoculum must produce the optimum growth (Balouiri et al. 2016; Thatai and Sapra 2016). Therefore, the inoculums should be prepared according to CLSI or EUCAST protocols (Fig. 7). Typically, the fresh microorganism strain culture is diluted to 0.5 McFarland $BaSO_4$ turbidity standard which corresponds to inoculum sizes of $0.5 \times (10^3–10^8)$ CFU/mL (Balouiri et al. 2016; Thatai and Sapra 2016).

Incubation temperature and time

Previous studies have demonstrated that depending on the antimicrobial compound and microbes tested, the MICs tend to increase with increasing incubation time and temperature (Misra et al. 2011; Balouiri et al. 2016; Thatai and Sapra 2016). The range of incubation temperature usually used to support maximum growth is 30–35°C, while the incubation time should give a stable growth. In general, the choice of incubation time depends on the growth rate of the pathogen to be tested. For example, the minimum incubation period reported to give a stable growth for bacteria is 18h, whereas for the fungi is 72h (3 days) (Thatai and Sapra 2016).

Determination of minimal inhibition concentration (MIC) endpoint

The most significant source of inter-laboratory variability is the MIC endpoint determination and antimicrobial susceptibility test (Thatai and Sapra 2016). For instance, in the case of

Table 1 Summary of various techniques used to assess the susceptibility of microorganisms (Varaldo 2002; Kemp et al. 2009b; Said-Galiev et al. 2011; Balouiri et al. 2016)

Antimicrobial susceptibility testing methods	Advantages	Disadvantages
Agar disc diffusion and broth dilution	Mostly used, easy to operate, highly standardized, sensitive, and recommended by the international standard guideline protocols	Not convenient for the rapid and effective detection of the antimicrobial activity of AMCs
Commercial automated system, flow cytofluorometric, and bioluminescence assay	Useful to obtain rapid results and better understanding of the effect of tested compounds on the viability and cell damage imposed to the tested microbes	Require the use of complex and specified equipment which need more evaluation for standardization and reproducibility
PCR-based techniques, whole-genome sequencing, and MALDI-TOFMS	Efficient for the rapid detection of the antimicrobial compound's effect	Not commonly used since they require the use of complex molecular techniques and specific equipment which are costly

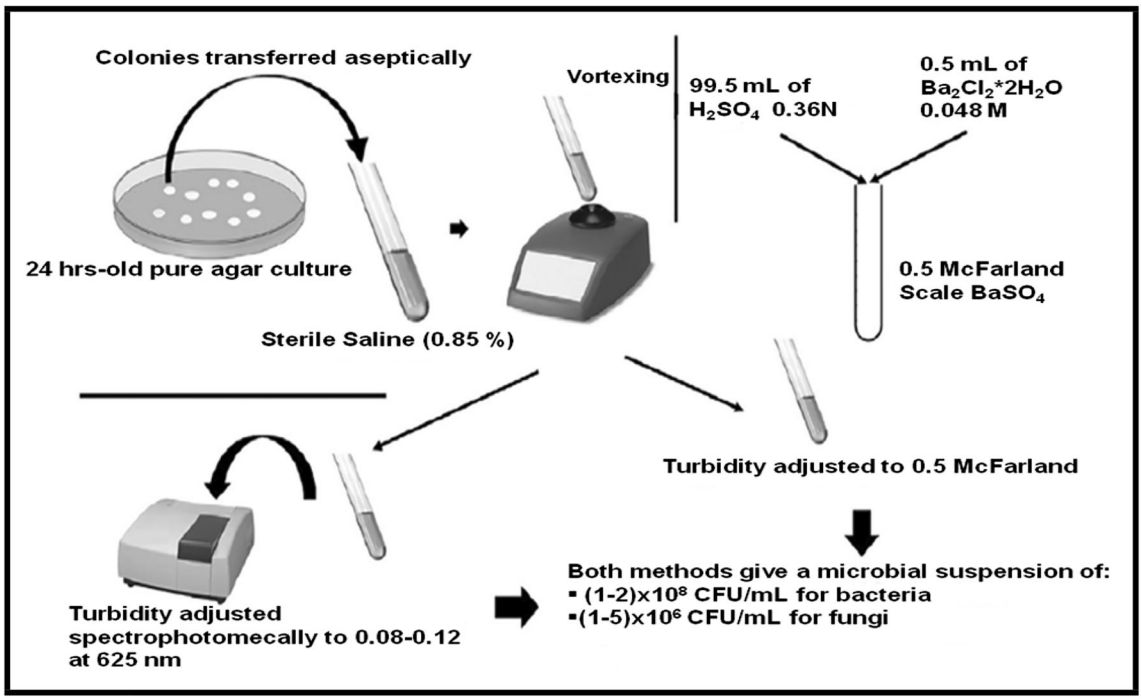

Fig. 7 Standard protocol recommended by CLSI for the preparation of microbial inoculums by direct colony suspension (Balouiri et al. 2016)

the MIC test, the determination of the optical density (OD) using a spectrophotometer, which is then converted to a percentage, was considered as an endpoint (Thatai and Sapra 2016). The addition of calorimetric indicator (also called a turbidometric assay, e.g., resazurin dye) into the culture medium has also been demonstrated to give better visual, accurate, and more evident endpoints (Fig. 8) (Elshikh et al. 2016; Thatai and Sapra 2016; Teh et al. 2017).

Antimicrobial activity mechanism of BINC materials

The antimicrobial activities of NPs, such as TiO_2, Cu, ZnO, Au, and Ag, have already been reported (Wang et al. 2012b; Gupta et al. 2013; Dizaj et al. 2015; Dong et al. 2015; Fosso-Kankeu et al. 2016; Pulit-prociak and Banach 2016). Among these NPs, the antimicrobial mechanism of Ag NPs is widely studied because Ag NPs have a broad spectrum of properties and antimicrobial activities (Dizaj et al. 2015). Previous studies have reported that the positively charged metal generally interacts with the negatively charged groups on microbial cell-wall through chemical interactions, Van der Waals forces, and electrostatic interactions to produce reactive oxygen species (ROS) as free radicals. These ROS are then responsible for oxidative stress, which then causes disruption of the membrane cell wall integrity for the microorganism and finally cell death (Elzinga et al. 2012; Hajipour et al. 2012; Domènech et al. 2013; Beyth et al. 2015; Dizaj et al. 2015; Fosso-Kankeu et al. 2016; Pulit-prociak and Banach 2016). For example, Fig. 9 illustrates the antibacterial mechanism of NPs.

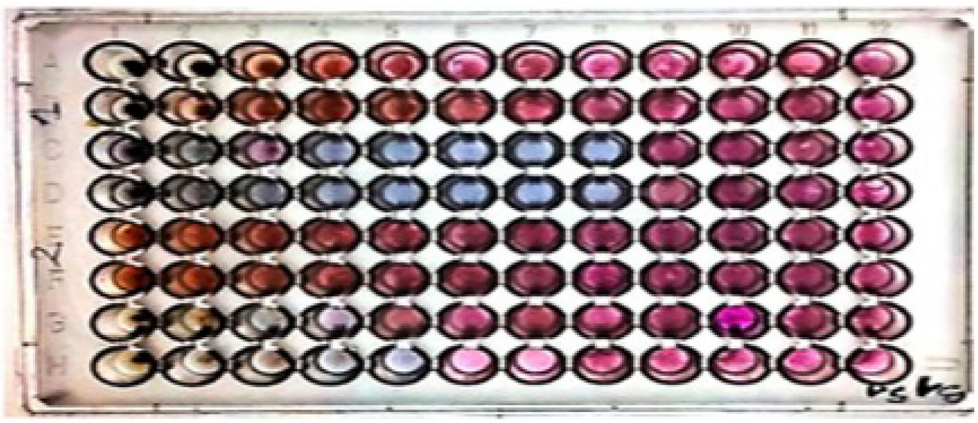

Fig. 8 Pictorial representation of calorimetric indicator (or assay) for MIC endpoint determination by broth microdilution (Balouiri et al. 2016)

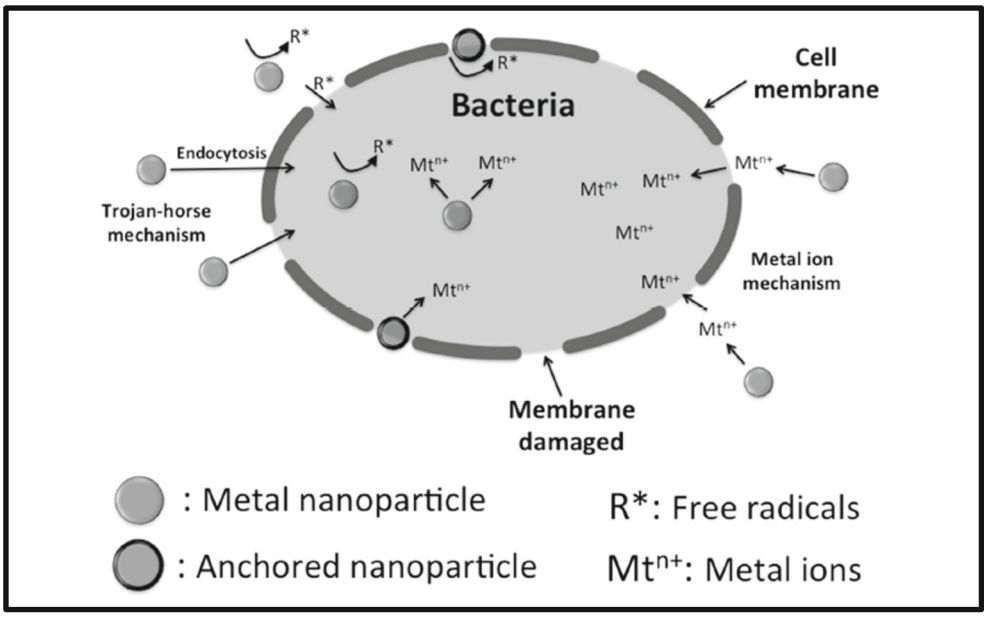

Fig. 9 Illustration of the nanoparticle's mechanism of bacterial inhibition (Palza 2015)

The mechanism of antimicrobial activity of carbon-based nanomaterial composites (e.g., polymeric nanocomposites) previously reported include first the physical and chemical interactions of the carbon-based NMs with the microorganism cell membrane and then the production of oxidative stress from the carbon compound which finally leads to cell membrane damage (Dizaj et al. 2015).

The microbial inhibition mechanism of polymers or BINCs is still not well known. From previous studies, it was assumed that the inhibition mechanism is mainly related to the release of metal NPs incorporated in the polymer nanocomposite and the electrostatic forces between the NPs (positively charged) and the bacteria membrane cell wall (charged negatively) (Cioffi et al. 2005; Anitha et al. 2012; Palza 2015; Baranwal et al. 2018; Dhanavel et al. 2018). Figure 10 gives a summary of the biocidal activity mechanism of polymer-inorganic nanoparticle composites. Table 2 presents a list of the various BINCs and their inhibition mechanisms.

The physicochemical properties of nanomaterials as factors affecting the antimicrobial activity mechanisms of nanomaterials (metal nanoparticles and polymer-metal nanoparticle composites)

The physical and the chemical properties of nanomaterials such as surface charge, type of functional groups present on NMs surface, particle size and particle length, crystal structure, and surface morphology are critical factors affecting the antimicrobial activity mechanism of nanomaterials (Domènech et al. 2013; Dizaj et al. 2015; Wang et al. 2017). Some of these physical and chemical properties of nanomaterials have been presented in Table 3.

Microbial properties affecting the activity of antimicrobial compounds

According to literature, the antimicrobial activity of engineered NMs has been reported to be due to the interactions of NMs with the microorganism cell wall membrane (Hajipour et al. 2012). These interactions are affected by inherent properties to microorganisms. These properties include cell wall membrane with differences in their compositions, growth rate, and formation of biofilm (Hajipour et al. 2012).

Composition of the membrane cell wall

The interaction of Gram-negative bacteria with a nanomaterial is different to that of Gram-positive bacteria because, in Gram-negative bacteria, the membrane cell wall includes a thin peptidoglycan (PG) layer, an outer membrane covering the surface membrane and lipopolysaccharides (Cabeen and Jacobs-Wagner 2005; Hajipour et al. 2012). The membrane cell wall of Gram-negative is generally chemically and structurally complex as well as resistant to hydrophobic compounds and detergents (Singleton n.d.; Hajipour et al. 2012). On the other hand, Gram-positive bacteria cell wall membrane is simple and consists of a thick layer of PG fixed to teichoic acid, which is unique to the cell wall of Gram-positive (Fig. 11) (Singleton n.d.; Scott and Barnett 2006; Hajipour et al. 2012).

Table 2 Various BINCs and their inhibition mechanisms

BINCs	Inhibition mechanisms	Microorganisms inhibited	References
Agarose-metal nanoparticle (Ag or Cu) composite films	Bactericidal	*Escherichia coli strain* DH5α	(Datta et al. 2008)
Cross-linked amylopectin/exfoliated titanate nanosheet-supported AgNPs (Cl-AP/exf.LT-AgNPs)	The mechanism of inhibition was bactericidal; it was based on the interaction of AgNPs with the bacteria cell membrane	*Escherichia coli* and *Bacillus subtilis*	(Sarkar et al. 2017)
Cellulose/silver nanobiocomposites	The inhibition mechanism was bactericidal against the bacteria but did not affect the growth of the fungi	Gram-negative bacteria and fungi	(Davoodbasha et al. 2015)
Chitosan/silver nanocomposite (CSNC) films	The mechanism of inhibition was reported to be due to the electrostatic interaction between the microorganism's membrane cell (highly negatively charged) and the CSNCs (positively charged)	Gram-positive strain (*Streptococcus pyogenes*, *Staphylococcus aureus*) Gram-negative strain (*Pseudomonas aeruginosa*, *Salmonella enterica*)	(Das et al. 2013)
Poly(butylene adipate-co-terephthalate (PBAT)/copper-NPs; PBAT/copper-cuprous oxide-NPs	The synthesized PBAT-based nanocomposites have shown a bactericidal activity against non-resistant bacteria strains	Non-resistant bacteria strains: *Enterococcus faecalis*, *Streptococcus mutans*, and *Staphylococcus aureus* Resistant bacteria: *Acinetobacter baumannii*	(Jaramillo et al. 2019)
Poly(lactic acid)/zinc oxide NPs (PLA/ZnO) Bionanocomposite films	The inhibition mechanisms resulted from the interaction between the negatively charged membrane surface of the bacteria and the positively charged surface of ZnO NPs, leading to the production of ROS which inhibit the growth of the bacteria	*Escherichia coli* and *Staphylococcus aureus*	(Kim et al. 2019)
Fibroin-silver nanocomposite (FSNC), Fibroin-gold nanocomposite (FGNC)	Both FSNC and FGNC have demonstrated significant inhibition activity against *Klebsiella. pneumoniae* and *Aspergillus fumigatus*	*Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Aspergillus fumigatus*	(Mane et al. 2017)
Chitosan-gold nanocomposite	The mechanism of inhibition was bactericidal	*Staphylococcus aureus*, *Pseudomonas aeruginosa*	(Regiel-Futyra et al. 2015)
Carboxymethylchitosan/alginate/copper (CMC/Alg/Cu)	The mechanism of antibacterial activity was significant in killing clinical bacteria (bactericidal)	Clinical bacteria	(Lu et al. 2018)
Poly(lactic)/TiO_2NPs	Bactericidal activity at higher concentration of TiO_2 (5wt%)	*Escherichia coli* and *Aspergillus fumigatus*	(Toniatto et al. 2017)

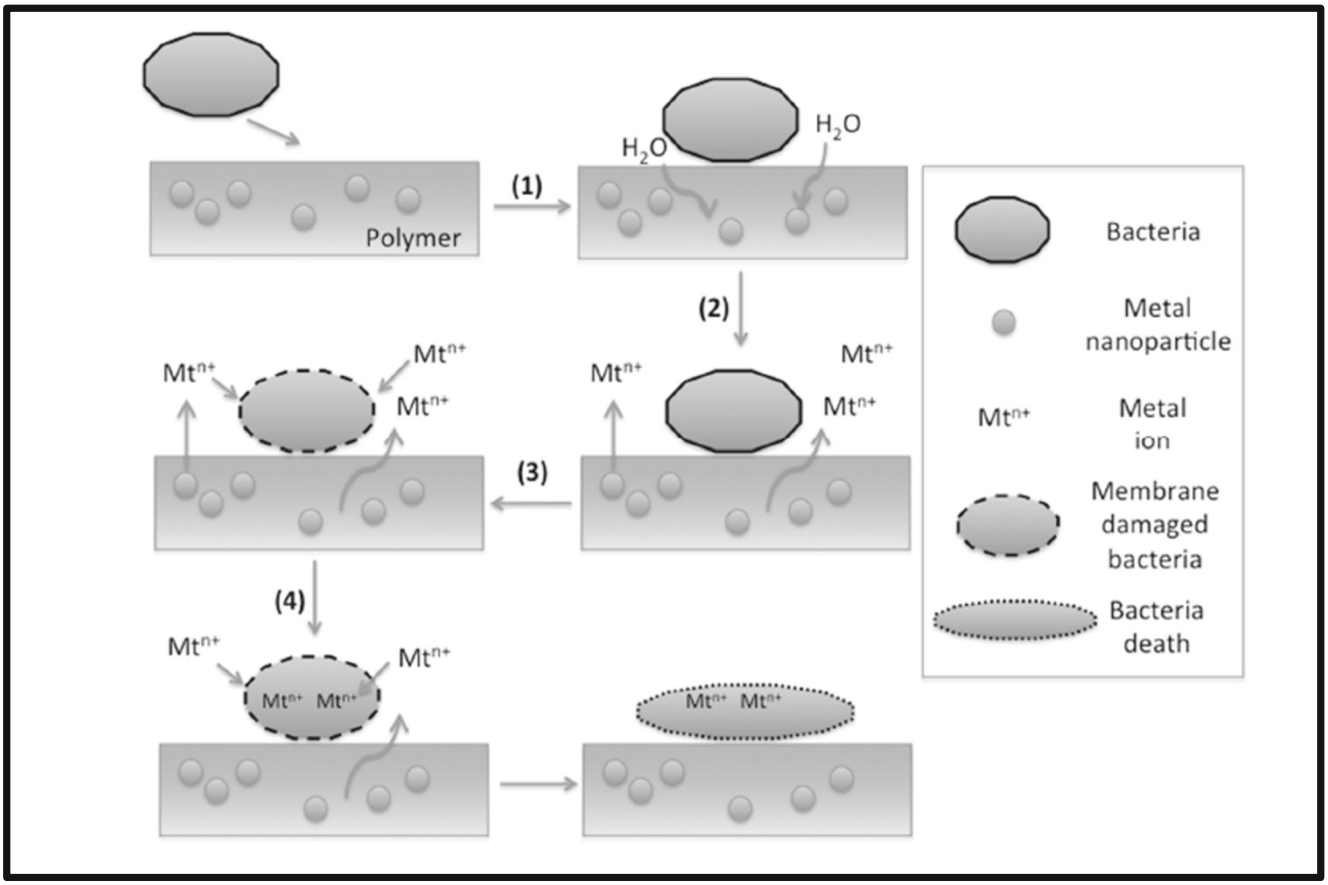

Fig. 10 Summary of antimicrobial inhibition mechanism of polymer-metal nanoparticle composites: (1) The microorganism (e.g., bacteria) is adsorbed on the surface of the polymer-metal nanoparticle composite, and due to the media around the bacteria, there is turn on of diffusion of water in the polymer matrix; (2) during the dissolution process, the metal NPs are released on the surface of the polymer nanocomposite; (3) metal NPs arrive at the surface of the polymer nanocomposite, and then metal ions attack the bacteria cell wall membrane; and (4) metal ions bind to the bacteria cell wall through electrostatic forces to cause the membrane cell wall damage, leading to cell lysis, penetration of metal ions into bacteria, production of ROS, and finally cell death (Palza 2015)

Growth rate of pathogens

Slow-growing pathogens have been reported to be less susceptible to antimicrobial compounds (AMCs) or antibiotic drugs than fast-growing pathogens (e.g., fungi developed slower than bacteria) which could be related to the expression of stress-response genes (Lu et al. 2009; Hajipour et al. 2012).

Formation of biofilm

Biofilm formation is a complex microbial group that appears by adhesion to a solid surface and by secretion of proteins, extra-polysaccharide, or DNA matrix which covers the group of bacterial cell (Hajipour et al. 2012; Beyth et al. 2015; Roy et al. 2018); biofilms had demonstrated significant issues because when they formed, they defend pathogenic microorganisms against the activity of AMCs (or antibiotic drugs), and this is, therefore, one of the primary sources of chronic infections expansion (Hajipour et al. 2012; Beyth et al. 2015; Hall and Mah 2017).

Formation of biofilm in water treatment network

Biofilm formation in the water treatment network is the outcome of many physical, biological, and chemical activities and transport of particles (with microorganism cells) to the wetted surface as well as the attachment of microorganisms to the surface (National Research Council (US) Safe Drinking Water Committee 1982; Siqueira et al. 2011). The development of a biofilm starts at the pipe wall with the adsorption and transport of solute organics. Then microbial cells are brought to the conditioned surface where they attach. The attached microorganisms absorb nutrients, produce biomass, and generate extracellular products. At any step of its formation, parts of biofilm peel away from the pipe surface and re-entrain

Table 3 Physical and chemical properties of nanomaterials

Factors	How it affects the antimicrobial activity	Dominant factor in antimicrobial activity?	References
Size (the definite and certain size)	Smaller NPs have larger specific surface area resulting in a higher chance of being in contact with and passing through the bacterial cell membrane than with larger nanomaterials	No	(Pan et al. 2013; Esfandiari et al. 2014; Gurunathan et al. 2014)
Shape	NMs with diverse shapes can produce varying levels of bacterial cell damage through interactions with periplasmic enzymes. The effect of NMs shape on bacterial inhibition is because of the side reactivity and surface area	Yes	(Chen et al. 2010; Actis et al. 2015)
Roughness	As the NPs increase in roughness, the surface area and size favor the uptake of bacterial proteins followed by a reduction in bacterial adhesion	No	(Rajakumar et al. 2012; Ben-Sasson et al. 2014; Sukhorukova et al. 2015)
Surface charge of NMs (determined by the zeta potential	Positively charged NMs will inhibit the bacterial growth through electrostatic interaction of the negatively charged bacterial cell membrane. Negatively charged or neutral NMs do not interact with the negatively charged bacteria. The antibacterial activity is also possible at higher concentration of negatively charged NMs, due to the molecular crowding	Yes	(Pan et al. 2013; Arakha et al. 2015; Fang et al. 2015)
Modification of NMs through doping	Doped NPs have been demonstrated to generate more ROS which is a result of greater damage of bacterial cells	Yes	(Peng et al. 2010; He et al. 2014; Guo et al. 2015; Mehmood et al. 2015; Sangari et al. 2015; Podporska-Carroll et al. 2017)

in the water flow (National Research Council (US) Safe Drinking Water Committee 1982; Siqueira et al. 2011).

Structure of the biofilm and its properties that promote resistance to AMCs

Biofilm is mainly made of a capsule called glycocalyx, which is highly adsorptive (can collect a large amount of detritus in natural water) because of its polyelectrolyte nature (Mah and O'Toole 2001). The combination of chemical and physical properties of biofilm helps to select the predominant microorganisms, which in turn modify the AMCs surface's environment (Bogino et al. 2013; Singh et al. 2017). The glycocalyx, since it forms an integral part of the structure of biofilm, is composed of a complex combination of exopolysaccharides (EPSs) DNA and protein in a self-produced extracellular polymeric matrix (Mah and O'Toole 2001). EPSs provide the architectural form of biofilms and stabilize their 3-dimensional structure. In the environment, around the interstitial voids, the biofilm has special architectural structures such as micro- and macro-colonies. The voids allow the flow of gasses, nutrients, and AMCs within biofilms (Bogino et al. 2013; Singh et al. 2017).

Besides, AMCs resistance of biofilm is caused by reduced diffusion of AMCs through the biofilm matrix, interaction of the AMCs with the biofilm matrix, genetic adaptation, levels of metabolic activity inside the biofilm, outer membrane structure, enzyme-mediated resistance, and efflux pumps (Singh et al. 2017). The resistance of AMC is maintained because of the change of colonies from exponential to slow or without growth. The glycocalyx matrix, through the enzymes and efflux system, inactivates AMCs and preserves the peripheral region of the biofilm. Remarkably, the cells starve for a particular nutrient and slow their growth in the intermediate position of a biofilm. Due to stress response to severe conditions, the surface-bound bacteria are prevented from cellular damage through the modifications at various gene expression levels (Singh et al. 2017).

Application and performance of AMCs in real wastewater solutions

It is always essential to provide a better understanding of whether the AMCs synthesized and tested in the lab have a reproducible performance when applied in real wastewater. In other words, this allows highlighting the factors related to the properties of real wastewater that can limit the performance of AMCs. From previous research studies, very few have addressed such a pertinent aspect of the implementation of AMCs; the reported studies mentioned that the antimicrobial compound synthesized and tested in the lab showed good performance when applied in real wastewater. However, their performances are limited by factors related to the properties of

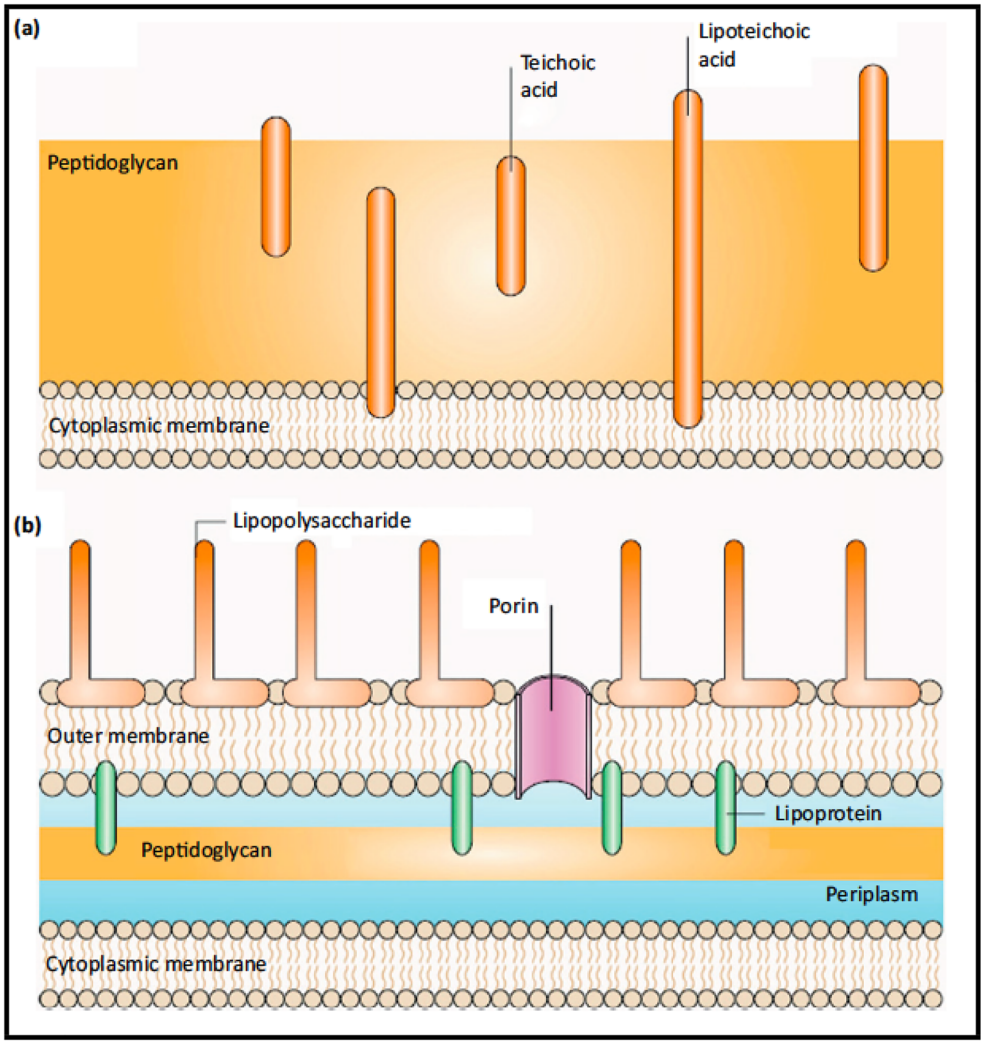

Fig. 11 Description of the bacterial cell wall membrane of a Gram-positive bacteria (**a**) and Gram-negative bacteria (**b**) (Hajipour et al. 2012)

real wastewater (Mcguigan et al. 2012; de Klerk et al. 2017), such as the type of nutrients and presence of organic and inorganic substances in the real water, which has been demonstrated to slow the disinfection process. For instance, a high concentration of chloride, phosphates, and sulfates ions in real wastewater has some detrimental effect on the efficiency of wastewater disinfection by AMCs (Mcguigan et al. 2012). According to Kong et al. (2010), the antimicrobial activities of chitosan and its derivative may also be affected by factors such as the pH, ionic strength, and temperature of wastewater (Kong et al. 2010). It has been reported that chitosan exhibits stronger inhibitory effect in an acidic environment, while a study by Tsai and Su showed that chitosan potency increased with increasing temperature (Tsai and Su 1999); on the other hand, increased concentrations of cations and anions in water have been found to affect the antibacterial efficacy of chitosan (Chung et al. 2003; Xing et al. 2009).

De Klerk and co-workers have also reported that the type of water medium can also affect the performance of AMCs due to the reduced amount of nutrients in real wastewater medium compare to an enriched nutrient broth medium. For instance, de Klerk et al. (2017) have shown that when using the real environmental wastewater samples, the inhibition of microorganisms by AMCs was higher than in nutrient broth media (de Klerk et al. 2017). Hence, the antimicrobial tests in the nutrient-rich media do not provide clear information of the performance of the tested AMCs because, in nutrient broth medium, the microorganisms can acquire enough energy in order to resist or to regenerate from the damage caused by the action of AMCs, while the environmental water lacks enough nutrients to retain the integrity of microorganisms after treatment with AMCs. Additionally, this higher inhibition of AMCs in environmental wastewater is also achieved if the sugar content in environmental wastewater is negligible (de Klerk et al. 2017). This is because the presence of sugar in wastewater can also favor the microorganisms to retain its integrity. De Klerk and co-workers have also shown that the presence of toxic metal ions in environmental wastewater is detrimental to the growth of microorganisms at high concentrations and may, therefore, bias the inhibitory activity of

AMCs. This is because these toxic metal ions can harm the bacterial cells (de Klerk et al. 2017).

Moreover, in the natural environment, the microorganisms have adapted and developed resistance; some of them carry antibiotic resistance genes (ARGs), especially extracellular antibiotic resistance genes. ARGs result from a mixture of antibiotics from farming, hospitals, industries, and agriculture. They are known as emerging micropollutants because they are toxic to the environment and human health (Iakovides et al. 2019; Li et al. 2019). Such microorganisms (ARGs) will be more resistant to the AMCs developed in the lab compared to the commercial strains which have been purchased for the antimicrobial test. Hence, it is recommended that after testing the AMCs using commercial stains, it will be beneficial to also apply the synthesized AMCs for the disinfection of real wastewater in order to obtain a better prediction of the synthesized AMCs antimicrobial performance under real conditions.

Conclusion

The different synthesis methods of BINCs have been reviewed. It was also noted that the in situ method was the most preferred method based on the reports from previous studies. The application of these emerging BINCs as disinfecting or antimicrobial agents was overviewed by elaborating on the different antimicrobial susceptibility testing methods. The antimicrobial activity mechanisms of these BINCs were also discussed; they have demonstrated long-term and strong antimicrobial activity. It is also important to note that biofilm may play a mitigating role in the potential of AMCs by affecting the disinfection or effective treatment of wastewater. Furthermore, it will be advantageous to complement the susceptibility testing methods by further testing of the inhibitory activity of AMCs in the natural environment of microorganisms; this approach will provide a better understanding of the AMCs performances in both enriched nutrient media and real environmental wastewater. To this end, the development of new nanostructured materials with higher antimicrobial performance and biocidal sensitivity toward resistant pathogenic microorganisms is a growing necessity and will benefit many researchers in overcoming the problem of wastewater treatment.

Acknowledgements The authors would like to acknowledge the National Research Foundation (NRF, Grant Number 120740), the Vaal University of Technology, the University of Johannesburg, and the North West University (Potchefstroom campus) for their valuable contributions to this work. Our heartfelt thanks to the Lord God Almighty for the wisdom and knowledge.

Availability of data and materials There are no data and materials for this review article.

Author contribution Anny Leudjo Taka: writing original draft, funding acquisition, project investigation, and management. Elvis Fosso-Kankeu: writing, reviewing, and editing. Xavier Yangkou Mbianda: writing, reviewing, and editing. Eliazer Bobby Naidoo: supervision, reviewing, and editing.

Funding National Research Foundation (NRF) "This work is based on the research supported wholly/in part by the National Research Foundation of South Africa (Grant Numbers: 120740)". This work is also partly supported by the Vaal University of Technology.

Declarations

Competing of interests The authors declare no competing interests.

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