Chapter 19

Nano-biotics: An Advanced Approach to Control Biofilm of Antibiotic Resistant ESKAPE Pathogens

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ABSTRACT

The rise of anti-microbial resistance in *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa and Enterobacter* species (ESKAPE) microorganisms present a significant worldwide general well-being risk. The development of biofilms, which is a pivotal component adding to the severity of numerous microbes, deteriorates the trouble of treating infections. Common antibiotics now and again demonstrate inadequate in wiping out biofilms because of their natural components of obstruction. Nano-biotics offer a confident way to deal with tending to infections connected with biofilms in this specific circumstance. Nano-biotics use nanotechnology to make innovative antibacterial substances that can successfully combat biofilm resistance mechanisms. Nano-biotics can enter the biofilm lattice, disrupt bacterial communication, and improve the transport of the antimicrobial medications to bacterial cells. This results in the viable anticipation of biofilm improvement and the elimination of previously existing biofilms. This Chapter features the most current improvements in nano-biotics for controlling the development of biofilms brought about by ESKAPE infections that are resistant to anti-microbials. It additionally discusses different nano-biotic approaches. Moreover, the review makes sense of how the combination of nano-biotics with conventional antibiotics or other therapeutic agents could help antimicrobial effectiveness and lessen the risk of resistance. Also, this chapter talks about the possible difficulties and future paths in the advancement and use of nano-biotics to treat diseases associated with biofilms.

KEYWORDS
Nano-biotics, Biofilm, Antibiotic resistant, ESKAPE, Pathogens

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INTRODUCTION

The term "nano biotics" refers to the administration of antibiotics, which are either wrapped in fabricated nanoparticles or that are chemically made and with 100-nm or smaller dimensions. These nano-biotics are designed to deliver modern antimicrobial medication directly to the microorganisms (Lei et al., 2018). The techniques can also be used in treating problems linked to planktonic and multiresistant biofilms (Batalha et al., 2019). Nano biotics is a revolutionary and thrilling way to administer antibiotics. Particles with a minimum diameter of 100 nm may be synthesized as pure antibiotics or in a mix of antibiotics and little particle (Saidykhan et al., 2016; Zhu et al., 2022). Antibacterial compounds are attached to particles. NPs may be chemically pure and have native properties; pure NPs covered with diverse groups of substances synthetically, such as polymers, citrates, and carboxylates (Mamun et al., 2021). Due to an inherent ability to target particular sites, nano-biotics penetrate more exactly and permanently through bacterial defense mechanisms than free antibiotic molecules (Lei et al., 2018). Nature cures might be developed with zinc oxide NPs, nickel, copper (Cu) NPs, titanium dioxide and silver NPs, and selenium lifetimes. However, nanoparticles with antimicrobial characteristics, referred to as "nano-biotics," can also be used to carry antimicrobial medications. In the form of liposomes and dendrimers and polymeric NPs, nano-biotics can be utilized to drug antimicrobials carriers. Nano-biotics become a tool to get drugs to patients through a precisely controlled spatial and temporal drug regimen. It increases the quantity of drugs that reach targeted tissue and pharmaceutical resistance. While nano-biotics also lessen drugstocks are on a downward path, they

sustained hazardous outcomes (Sánchez-López et al., 2020).

Nanoparticles may be a successful transporter for increasing and assisting to enhance the activity of antibiotics. Owing to their small dimensions and a greater source area to weight rate, nanomaterials are distinctive. This offers enhanced touch with germs and application that is controlled and shifts due to their condensed size and greater exterior area to mass rate. The survivability of nanoparticles NPs may be enhanced by altering its sizes, contours, and chemical constitution. By incorporating modified metallic, organic, biomolecular, radioactive, or antibody parts, nanoparticles NPs can efficiently destroy bacteria by a diverse range of approaches (Fig. 1) By including additional traits such as light, ionizing radiation, magnetic fields, and ultrasound, it might be further enhanced (Chakraborty et al., 2022).

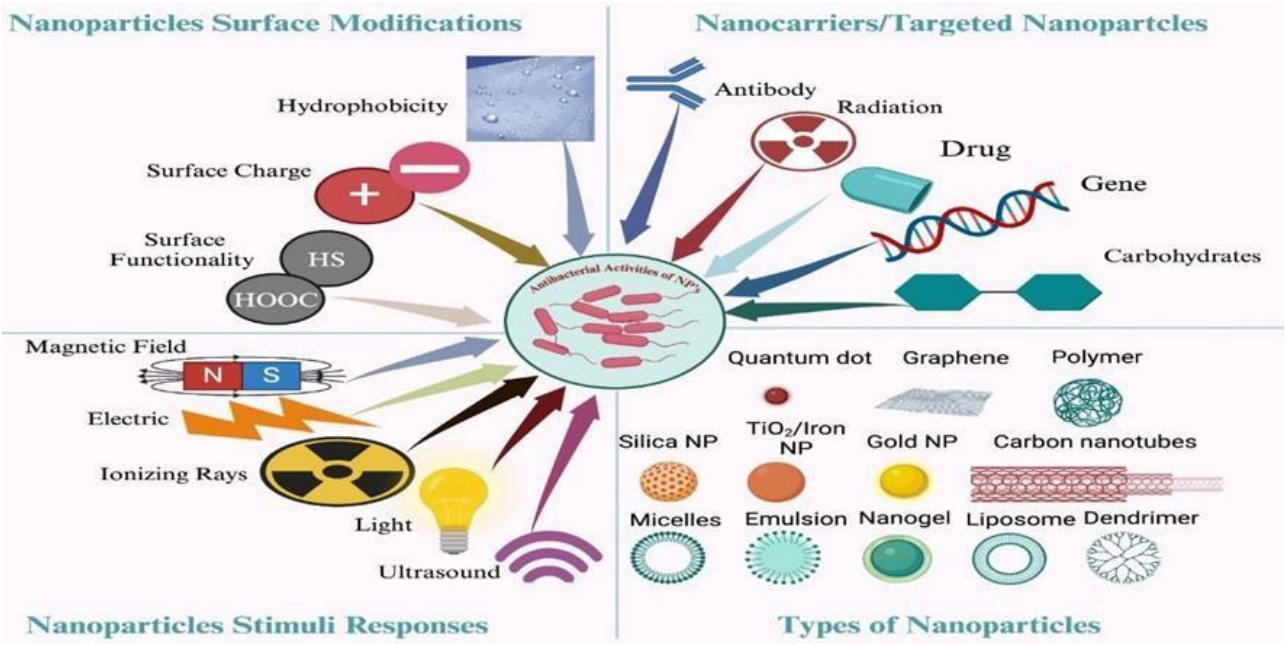

Fig. 1: Nano-biotics against AMR.

ESKAPE Pathogens

Another hurdle to achieving and paying for socialized health care is the challenge of the sustainable development goals. To address the issue of antibiotic resistance, the World Health Organization in 2017 worked out scripts for new therapies and produced a full worldwide list of artificially resistant small organisms (Slavcovici et al., 2015). The usage of reserve preparations to treat illnesses with first-line resistance will be necessary. These medications have a better economic performance and a reduced level of security (Santajit and Indrawattana, 2016). ESKAPE germs is an abbreviation for six multidrug-resistant bacteria that have a substantial impact on hospital-acquired infections/various illness in immunosuppressants. These bacteria have developed ways to evade the germicidal action of pharmaceuticals (Gheorghe et al., 2017). This abbreviation is gotten from *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii, Pseudomonas aeruginosa*, and *Enterobacter* species belong in the gram -positive and gram-negative bacterial category (Rice, 2010). Based on the results of the multicriteria assessments, the list was divided into groups, medium, high, and critical (Denissen et al., 2022). Critical include *Acinetobacter baumannii* carbapenem-resistant, *Pseudomonas aeruginosa* carbapenem-safe, *Klebsiella pneumoniae* third generation cephalosporin-resistant, *Enterobacter spp*. third-generation cephalosporin-resistant. High classification include *Enterococcus faecium* vancomycin-resistant, *Staphylococcus aureus* methicillin-resistant, vancomycin intermediate and resistant (Pendleton et al., 2013).

Antimicrobial Resistance Mechanisms of ESKAPE Pathogens

The bacterial chromosome, transposon and plasmid act as a carrier of antimicrobial resistance gene (Giedraitiene, et al., 2011). The main causes of antimicrobial resistance are the binding sites of drug modified, inactivation of the drug, formation of biofilm, drug efflux (Wright, 2005).

Vancomycin-Resistant *Enterococci Faecium*

It is deeply troubling that enterococci, and notably vancomycin-resistant *Enterococcus*, are showing alarming rates of antibiotic resistance. (Cencič and Langerholc, 2010), which is primarily concomitant with *E. faecium* (Hope et al., 2008). VRE has a six types (Van A-E, Van-G). Van-A is the most common and has the highest degree of resistance to all glycopeptide antibiotics (Smith, 2004). In 2011, Scientists provided evidence of two distinct clades (A, B) of *E. faecium* that exhibit genetic differences. Both clades exhibit the presence of low-affinity binding proteins for penicillin (referred to as PBP5) that

have a weak binding capacity to β-lactam medicines. Furthermore, clade B has a gene that encodes ampicillin-sensitive PBP5 (pbp5S), whereas clade A possesses numerous resistance genes and virulence determinants due to IS16 and a gene that encodes ampicillin-resistant PBP5 (pbp5R) (Arias and Murray, 2012).

Methicillin-resistant *Staphylococcus aureus* (MRSA)

Staphylococcus infections were effectively treated with penicillin previously. However, the overuse of these antibiotics in 1948 resulted in the appearance of *Staphylococcus* isolates that produce β-lactamase. Penicillin G resistance investigated in 65–85% Staphylococcus clinical isolates (Bush and Bradford, 2013). The primary antibiotics used to treat MRSA infections are glycopeptide antibiotics, such as teicoplanin and vancomycin. (Hryniewicz and Garbacz, 2017).

Klebsiella pneumonia

The β-lactamase enzymes evolved from the Klebsiella pneumoniae strains degrade the β-lactam antibiotics chemical composition such as carbapenems, cephalosporins, penicillins. The rising frequency of *K. pneumoniae* resistant to carbapenem poses substantial issue for clinicians due to the typical use of carbapenems for the treatment of infections (Queenan and Bush, 2007).The *K. pneumoniae* super enzyme, NDM-1 led to a higher number of *K. pneumoniae* isolates that are resistant to carbapenem antibiotics. This might potentially endanger the effectiveness of other antibiotics such fluoroquinolones, aminoglycosides, and β-lactams (Yong et al., 2009). *Acinetobacter baumannii*

There have been recent reports of the appearance of *A. baumannii* strains that produce carbapenemase and contain imipenem metallo-β-lactamases, which are carried by certain genes and blaOXA gene carry oxacillinase serine β-lactamases. Both colistin and imipenem are not effective against these germs, and they are able to resist most conventional antibiotics due to combination of resistance genes.(Vila et al., 2007).

Pseudomonas aeruginosa

Multiple strains of *P. aeruginosa* have inherent reduced susceptibility to various antibacterial drugs, together with a tendency to acquire resistance while undergoing treatment, particularly in strains that are resistant to carbapenems, namely imipenem. *P. aeruginosa* changes the porin structure and synthesize the chromosomal *AmpC* to combat imipenem drug. The lower amount of AmpC enzyme alone do not cause resistance to carbapenem drugs because they have limited ability to break down these drugs. However, when there is an overproduction of *AmpC* enzymes, combined with reduced permeability of the outer membrane porin and/or overexpression of efflux pumps, it contributes to high-level resistance to carbapenem drugs in this pathogen (Elsner et al., 2000). *P. aruginosa* can produce extended-spectrum beta-lactamases (ESBLs), carbapenemases and imipenem metallo-β-lactamases to resist antibiotic mechanisms. The presence of these enzymes results in a significant increase in carbapenem resistance in *P. aeruginosa* isolates. Additionally, it may lead to the development of strains that are resistant to fluoroquinolones, since the same plasmid may carry the mechanisms of resistance for both types of antibiotics (Livermore, 2002).

***Enterobacter spp*.**

Enterobacter microbes species have carbapenemases, like OXA,VIM, KPC, metallo-β-lactamase-1 and Expanded Range Beta-Lactamases (ESBLs) (Castanheira et al., 2011). Besides, it is critical to accomplish steady concealment of *AmpC* β-lactamases and upregulated by genetic mutation in this group of microrganisms. The MDR strains display resistance ta practically all current antimicrobial drugs except colistin and tigecycline (Boucher et al., 2009).

Nanotechnology Applications in Medicine and Antimicrobial Therapy

The area of nanotechnology and the discoveries connected with nanomedicines are broad and envelop a wide range of applications. Nanomedicine has encountered amazing advancement, raised the efficacy of medications, and prompted significant enhancements in healthcare results. It is important to analyze the striking limits of nanotechnology in the field of healthcare (Keskinbora and Jameel, 2018). Because of their small size, nanoparticles have a high surface-to-volume ratio, which permits them to rapidly absorb a lot of drugs as they circulate through the circulation system. Their extraordinary properties originate from their increased surface area, which improves their mechanical, magnetic, optical, and catalytic properties and makes them more reasonable for use in pharmaceutical applications (Huang et al., 2018; Ren et al., 2021). Nanomedicines have been used in gene therapy too. Multiple inquiries have concentrated on the use of viral vectors as potential mechanisms for delivering medicine (Quader et al., 2021).

Nanomedicine is used in many different fields, such as drug delivery, diagnostic and imaging tools, high-performance screening platforms, antimicrobials, vaccine research, wearable technology (Wang et al., 2021). The primary and most significant antibacterial mechanisms of nanoparticles involve the generation of reactive oxygen by photocatalysis. The mechanisms by which reactive oxygen species (Hope et al. 2008) exert their effects include the breakdown of bacterial and cell membranes, disruption of energy transfer, reduction of enzyme activity, and suppression of DNA synthesis (Fig. 2) (Kianfar and Kianfar, 2019).

Mechanism of Action of Nanobiotics against Biofilms

Multiple studies have documented nanoparticles (NPs) ability to reduce biofilms formation caused by various

bacterial pathogens (Ahmed et al., 2016). NPs have an anti-biofilm effect because they can penetrate bacterial cell walls and membranes, interfere with the biofilm's EPS matrix, and disrupt the quorum sensing system (Gupta and Chhibber, 2019) (Sánchez-López et al., 2020). Reactive oxygen species activity of NPs cause membrane damage in the pathogen (Li et al., 2012). Hydrogen peroxide, hydroxyl radicals, and superoxide radicals are produced by ROS mediated oxidative stress. These radicals cause protein degradation and DNA damage leading to cell death of bacteria (Fig. 3) (Chhibber et al., 2017).

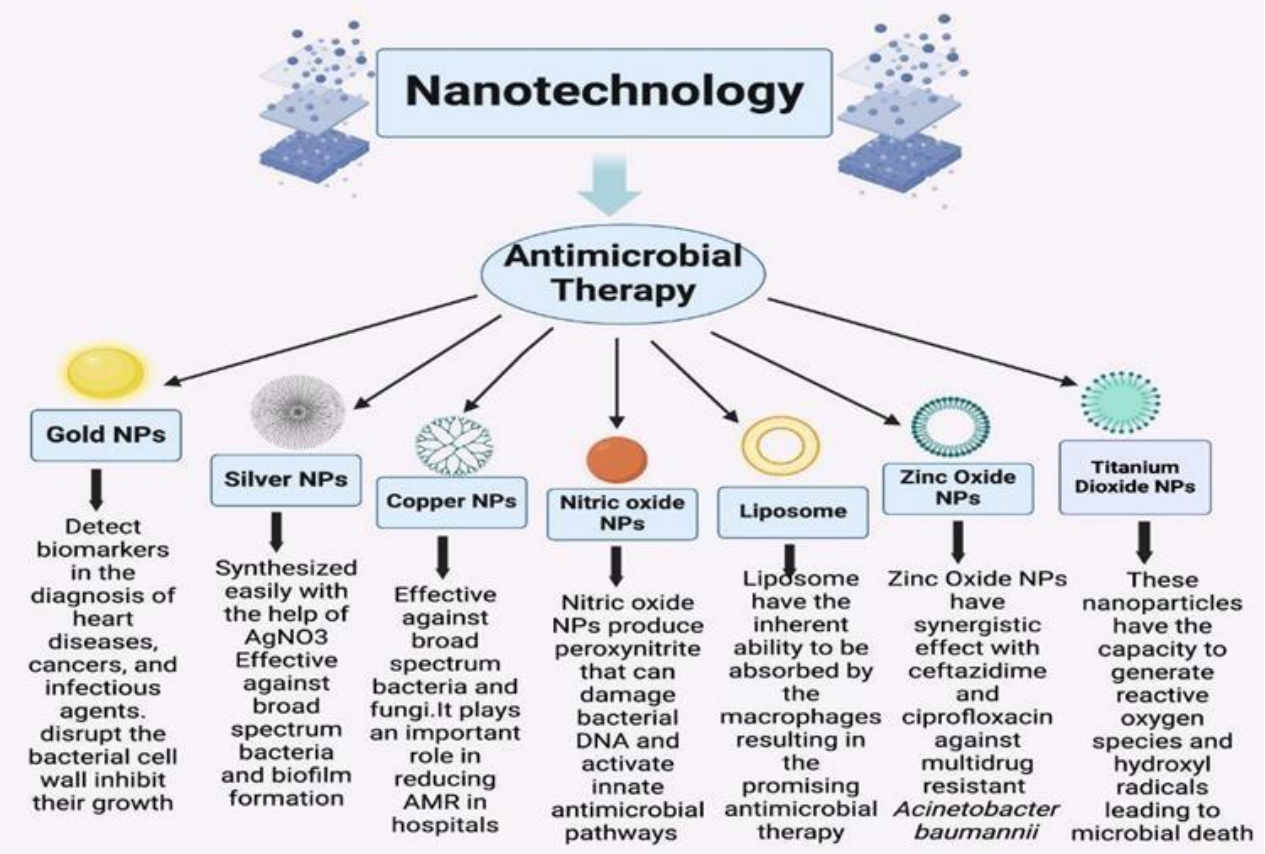

Fig. 2: Applications of Nanotechnology in healthcare

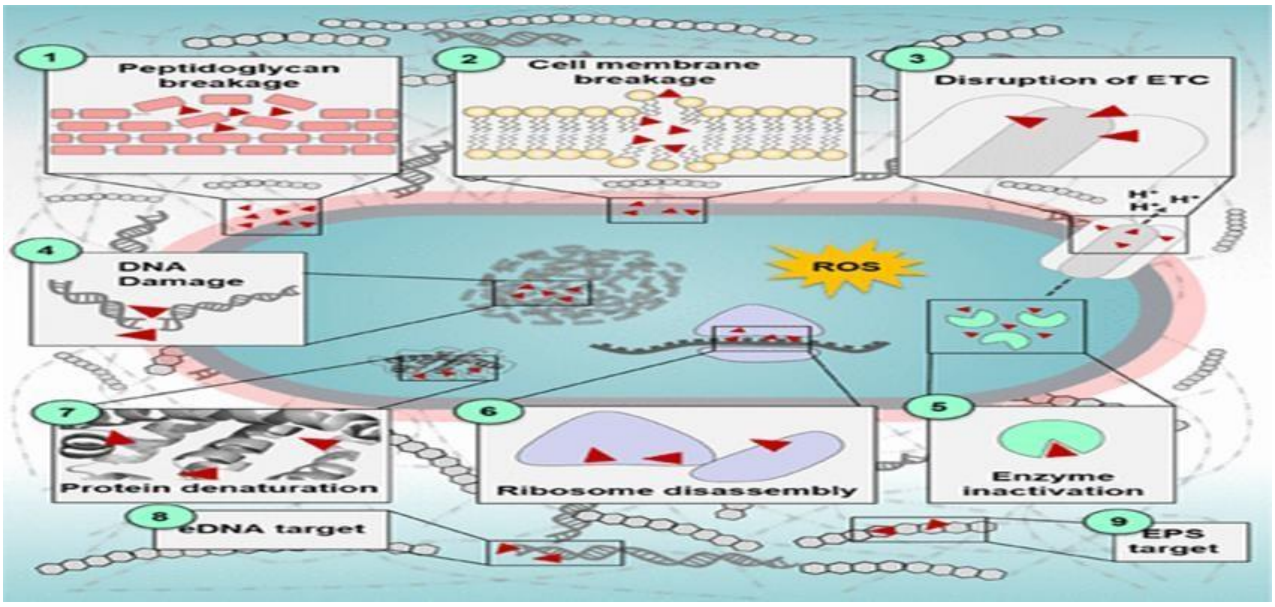

Fig. 3: Antibiofilm Mechanism of Nanoparticles

Synergistic Approaches with Conventional Antibiotics

In recent years, nanotechnology has become a significant area of study with diverse applications across several

sectors. The chemical and physical properties of nanoparticles are influenced by high surface area to volume ratio at nanoscale, resulting in distinct properties compared to bulk matter (Amendola, 2008). The unique characteristics of nanoparticles make them appealing choices for a variety of applications. Silver nanoparticles have been widely studied for their antibacterial ability in very recent times. The growth of bacilli resistance and the dissemination of numerous resistant strains demonstrate an urgent need for such compounds. Given the global resistance of antimicrobial drugs, the utilization of silver nanoparticles as new antimicrobial agents for fighting infections is of particular relevance (Yuan et al., 2017). Recent scientific investigations have shown that combining silver and appropriate antibiotics can improve the antimicrobial properties of the drug against some pathogenic bacteria (Nishanthi et al., 2019). Comprehensive and in-depth studies on the impact of SACs on multidrug-resistant bacteria, particularly ESKAPE pathogens, are lacking The study conducted by (Mishra et al., 2024) explored how SACs combat bacteria like ESKAPE strains found in food sources, like chicken, beef, fish, and sprouts. The research also aimed to know about the antibacterial mechanisms of these SACs. The study examined the use of anti-microbial (SACs) and silver nanoparticles in combination to fight ESKAPE germs using a two-dimensional checkerboard method. The combination of AgNPs and antibiotics kills the multidrug-resistant ESKAPE bacteria more effectively. In both the log stage and the biofilm development stages, the SACs demonstrated antibacterial activity against cells.

Table 1: Nanoparticles characteristics, activity, and mechanism of action against multidrug-resistant (MDR) bacteria

Nanoparticles	Size	Targeted bacteria and antibiotic resistance	Mechanism of action	Factors affecting antimicrobial activity	References
Magnesium Oxide (MgO)	15-100nm	*E. coli, S. aureus*	Alkaline effect, ROS production, lipid peroxidation	Concentration, pH, particle size	(Rudramurthy, et al., 2016)
Titanium Dioxide (TiO_2)	35-45nm	*P. aeruginosa, S. aureus, E. coli, E. faecium*	Surface adsorption, ROS production	Shape, crystal structure, Size	(Hemeg 2017), (Rudramurthy, et al., 2016)
Zinc Oxide (ZnO)	10-100nm	*E. coli, K. pneumoniae, K oxytoca E. aerogenes ,*	Disruption of membrane, ROS production,protein damage, surface adsorption	Concentration and particle size	(Hemeg 2017), (Rudramurthy, et al., 2016)
Iron Oxide	1-100nm	MRSA, *E. coli, K. pneumoniae*	ROS mediated oxidative stress: hydrogn peroxide, hydroxyl, and superoxid radicals	Loss of dispersity and magnetism due to air oxidation, high chemical activity	(Rudramurthy, et al., 2016), (Zaidi et al.,2017)
Aluminium (Al)	10-100nm	*E. coli*	ROS causes disruption in cell wall		(Rudramurthy, et al., 2016)
Silica (Si)	20-400nm	MRSA	ROS cause disruption in cell wall	Shape, stability particle, size	(Zaidi et al.,2017)
Copper (Cu)	2-350nm	*A. baumannii, E. coli*	ROS generation, DNA damage, protein oxidation, lipid peroxidation,	Concentration, particle size	(Zaidi et al.,2017), (Hemeg 2017)
Gold (Au)	1-100nm	MRSA	Membrane damage, ATPase activity reduced, respiratory chain disruption, membrane potential loss	Particle size, roughness,	(Zaidi et al.,2017), (Hemeg 2017)
Silver (Ag)	1-100nm	ESKAPE pathogens	ROS production, DNA, and protein damage, respiratory chain disruption, lipid peroxidation	Shape and size	(Hemeg 2017), (Rudramurthy, et al., 2016)

Several theories have been put out to explain how AgNPs exert their effects (Zhao et al., 2023). AgNPs cause a continuous and persistent ion release when they penetrate bacterial cells, which leads to the consumption of ATP, condensation of DNA, generation of ROS, and eventual death. While the specific process via which the antibacterial effectiveness of SACs is enhanced remains unclear, earlier studies indicate that the presence of silver nanoparticles in SACs results in a more confined release of Ag+ ions, which leads to the destruction of bacterial cells. Silver ions produced are harmful since they interact with and damage cell wall proteins of bacteriaa and DNA, resulting in cell death (Deng et al., 2016). A different investigation on the pharmacodynamic interaction between AgNPs and antibiotics suggested that the combinations impair the bacterial membrane, enhance K+ ion release, and prevent biofilm formation (Thirumurugan et al., 2016). Similarly, further research revealed that antibiotics and AgNPs together have been shown to have combined effects against *P. aeruginosa, S. aureus,* and *E. coli.* (Saha et al., 2007). Furthermore, at doses much below the minimum inhibitory concentration (MIC) of either the nanoparticle or antibiotic component of the combination, AgNPs coupled with antibiotics demonstrated increased antibacterial efficacy against multi resistant, β-lactamase and carbapenemase-producing Enterobacteriaceae (Panáček et al., 2015). Saha et al. describe on the direct attachment of ampicillin, streptomycin, and kanamycin to gold nanoparticles (Saha et al., 2007). The complexes obtained exhibited a reduced minimum inhibitory concentration (MIC) compared to the free drug equivalents when tested against both Gram-negative and Gram-positive

bacteria. The authors of the study did not provide a detailed explanation of the mechanism behind these effects. However, Fayaz et al. conducted research to understand how their gold nanoparticles functionalized with vancomycin showed activity against strains that are typically resistant to vancomycin due to either mutations (vancomycin resistant *S. aureus*) or membrane structure (*E. coli*) (Fayaz et al., 2011). The proposal suggests that the antibiotic can only lead to nonspecific, multivalent contacts and attachment of the carrier to the cell wall synthesis proteins when it is complexed with the NPs. The scientists deduced that the weakened integrity of the cell membrane and the resulting cell death were the consequences of non-specific binding, as detected using transmission electron microscopy, due to the existence of pits in the cells (Gao et al., 2018). Gold nanoparticles (NPs) have been shown to exhibit synergistic effects when used in conjunction with several antibiotics against susceptible bacteria, including both Gram-negative and Gram-positive bacteria. An example of notable synergy is shown when gold nanoparticles are used with meropenem to combat Acinetobacter baumannii (Shaker and Shaaban, 2017) while amoxicillin and streptomycin show similar effects against Staphylococcus aureus and Escherichia coli. These effects were seen at concentrations of gold ranging from 1 to 16 mg L−1. For bismuth nanoparticles (NPs), the assessment of synergistic effects has been evaluated using antibiotics that hinder the production of nucleic acids, namely fluoroquinolones. Only the combination of ciprofloxacin and bismuth NPs demonstrated an increase in antibacterial efficacy against Klebsiella pneumoniae (Tarjoman et al., 2015). Zinc oxide nanoparticles (NPs) at concentrations ranging from 30 to 80 mg L−1 have been synergistically combined with fluoroquinolones (norfloxacin, ofloxacin) (Namasivayam et al., 2015) or β-lactams (cephalexin, ceftriaxone, cefotaxime) (Bhande et al., 2013) to significantly augment their efficacy against both Gram-positive (Staphylococcus aureus) and Gram-negative (Escherichia coli) bacteria. Research has investigated the use of Titanium dioxide nanoparticles (NPs) as an antibacterial agent in conjunction with streptomycin. The results have shown enhanced antibacterial effects against *Klebsiella pneumoniae, Salmonella typhimurium, Escherichia coli, and Staphylococcus aureus.*

Novel Nanomaterials and their Applications

Nanomaterials have been developed and examined to inhibit biofilm formation. There are two main categories into which they fall:

(i) Natural NPs encompass various types, such as solid lipid NPs, cyclodextrins, dendrimers, liposomes, and polymeric NPs.

(ii) Inorganic NPs such as quantum dots, fullerene, organic-inorganic hybrid, metallic (gold, silver, silica, copper, and iron) and their oxides (aluminium and iron oxides).

The bactericidal activity can be attributed to the characteristics of the nanomaterial or nanocarrier. Some enable the encapsulation of a medicine, shielding it from enzymatic inactivation, unfavorable environmental conditions (low pH or oxygen), bacterial defense mechanisms (Prateeksha et al., 2019; Singh et al., 2021).

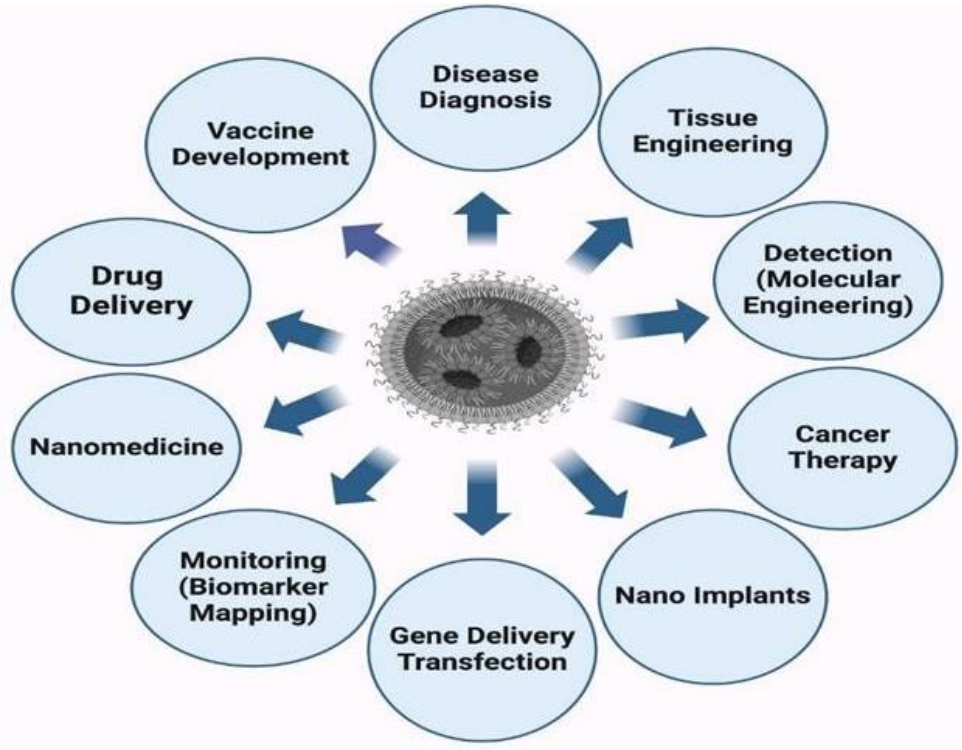

Fig. 4: Antibiofilm activity of nanomaterials

To investigate the capability of gold nanoparticles (AuNPs) to prevent the formation of biofilms, gold nanorods (AuNRs) were modified with a polymer called polymethacrylate. This polymer has carboxyl betaine groups and can vary its surface charge in response to near-infrared (Mamun et al.) Light and changes in pH. The reversible addition-fragmentation chain transfer polymerization (RAFT) reaction was used for the modification of the AuNRs. All the planktonic cells were killed off due to the charge fluctuations of pH responsive. Photothermal near-infrared (Mamun et al.) Irradiation induces localized overheating, resulting in the demise of *S. aureus* bacteria and subsequent disintegration of biofilm (Qiao et al.,

2020). The scientist examined a developed formulation of solid-lipid nanoparticles loaded with silver sulfadiazine (SSD-SLNs) which enhanced its efficacy in treating burn wounds associated with *P. aeruginosa* biofilm by incorporating DNase-I and chitosan gel. The vulnerability of *P. aeruginosa* biofilms to antibiotics enhanced due to DNase- I which breaking down the extracellular DNA (eDNA), resulting in the eradication of the biofilm. After 72 hours, Pure SSD and SSD-SLN could eliminate only 58.1% and 78.7% of the biofilm, respectively. The incorporation of DNase I into SSD-SLN inhibited 96.8% biofilm formation of *P. aeruginosa*. The SSD-SLNs and DNase-I synergism healed the wound completely within 21 days according to in-vivo study (Patel et al., 2019).

The silver NPs synthesis functionalized with α-Amylase (biofilm-disrupting enzyme) and polydopamine (DA) for inhibiting the growth of *S. aureus* biofilms on titanium surfaces (Tran, et al., 2021). A1 (Serpin), an elastase inhibitor, and LL37 (the endogenous host defense peptide) are administered topically by nanomedicine to treat chronic wounds by regulating their sustained release. The co-administration of A1 and LL37 successfully healed the injury in BJ fibroblast cells and keratinocytes, eliminated microbial contamination, suppressed E. coli and *S. aureus* biofilm development, and improved anti-inflammatory action (Fumakia, et al., 2016). The researchers developed a formulation of ofloxacin-loaded solid lipid nanoparticles (SLN) to treat *S. aureus* and *P. aeruginosa* biofilm related-pulmonary infections. This formulation exhibited selectivity and supported the arrival of the medication at the targeted spot, and increased effectiveness. The nano composite diminished threefold minimum inhibitory concentration (MIC) when compared with free antibiotics (Rodenak-Kladniew et al., 2019). As indicated by another study, oxacillin-loaded nanostructured lipid carriers (NLC) formulation upgrades the adequacy of oxacillin against MRSA (Alalaiwe et al., 2018).

Challenges and Future Perspectives

The formation of biofilms by ESKAPE infections has become challenging to treat because of the ineffectiveness of traditional treatments. The limited effectiveness of antimicrobials in biofilms is mostly attributed to their low penetration, enzymatic inactivation, and destruction inside the biofilm microenvironment. Nanotechnology become new and successful approach for treating bacterial biofilms with precision. Nanomaterials may effectively penetrate biofilm EPS matrix becauseof their minute size. This allows them to eradicate the sessile and persister cells due to their inherent antimicrobial properties and/or by administering therapeutic agents. Nanoparticles impede and disrupt the development of biofilms by compelling with the quorum sensing (QS) pathway and degrading extracellular polymeric substances and extracellular DNA (eDNA). Despite many benefits of nanotechnology, it is not without limitations. These include cytotoxicity, impacts on metabolism, inadequate renal clearance, and aggregation in the blood and protein cells, which might affect human health. The metal ions from waste nanoparticles also develop harmful effects in the body. Moreover, overcoming biological obstacles and evading immunity to accomplish precise delivery pose major challenges. It is necessary to identify and address the unknown adverse effects and costly clinical studies. To develop suitable antimicrobial nanomaterials, it is essential to have accurate in vivo and in vitro models that specifically address biocompatibility, antibiofouling, antibacterial, and nanotoxicology properties. To advance the clinical development of nanomaterials, it is mandatory to conduct a comprehensive investigation on basic, pharmacological, and biological characteristics of nanoparticles, as well astheir interactions with pathogens. Future work has the potential to solve all these limitations of nanomaterials. Thus, we conclude that Nano biotics offer a promising solution for addressing ESKAPE pathogens. This is achieved through the administration of therapeutics at sub-inhibitory doses and employing multiple bactericidal pathways to eliminate bacteria.

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