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Review on the Multi-Drug Resistant (MDR) Bacteria Associated with Nosocomial Infections and Development of Emerging Therapeutics

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Abstract
Multi-drug resistance (MDR) by certain group of bacteria associated with hospital acquired infections (HAI) represents a growing diagnostic and treatment challenges of infectious diseases globally. It poses a majority of the problems in the management of health in the health care facilities worldwide; this is in terms of efficacy and effectiveness thereby undermining efforts of the health care providers like World Health Organization (WHO) in curtailing the emerging and re-emerging diseases of public health significance. Multi-drug resistance (MDR) is caused as a result of mismanaging the antibiotics since time immemorial, this inappropriate use of antibiotics, especially broad-spectrum antibiotics, leads to the emergence and spread of antimicrobial resistant bacteria which led to the selection of highly resistant bacteria pathogens in the health care facility settings. Nosocomial infections, particularly those caused by MDR bacteria are often very complicated to treat, leading to various side effects, including prolong hospital stay and higher cost of treatment which affect the natural human microbiome. In the same vein, the development of new antimicrobial agents is lagging with few new ones underway. Therefore, searching for the new alternatives to treat nosocomial infections may help to overcome the multidrug resistance challenges by bacterial pathogen. Currently, development of new therapeutic agents to find the lasting solution to multi-drug resistance by nosocomial pathogenic bacteria is ongoing through modification of existing drugs, use of novel metal-based complexes, antimicrobial peptides, and antisense antimicrobial therapeutics.

Keywords: Antimicrobials, Bacteria, Multi-Drug, Nosocomial, Resistance.

INTRODUCTION
Nosocomial infections (hospital acquired infections) also known as hospital associated infections (HAIs) consist of a greater proportion of causes of death rate worldwide, being related with a substantial increase in prolonged hospital stay and treatment costs. According to the European Centre for Disease Prevention and Control (ECDC), a total of 8.9 million HAIs occur each year in European acute care hospitals and long-term care facilities (Sursten *et al.,* 2018). The populations with increased risk of infections are patients in the intensive care, surgical, oncology/hematology, burn units, and those undergoing organ transplant and neonates (WHO, 2018). The most common nosocomial infections are catheter-associated urinary tract infections (CAUTI), surgical site infections (SSI), central line-associated bloodstream infections (CLABSI), ventilator-associated pneumonia (VAP), and Clostridioides difficile infections (CDI) (Stygal *et al.,* 2020). Several sources of bacterial nosocomial infections

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have been described, including surgery procedures and invasive devices such as catheters and ventilators (Stygal *et al.,* 2020).

Many pathogenic bacterial species were found to be related with hospital acquired infections. The most common being caused by methicillin-resistant, vancomycin-intermediate and vancomycin-resistant *Staphylococcus aureus Burkholderia cepacia*, *Acinetobacter baumannii*, *Clostridium difficile*, *Pseudomonas aeruginosa*, *Clostridium sordellii*, *Mycobacterium tuberculosis*, and Non-Tuberculous Mycobacteria (NTM), Carbapenemase-producing Enterobactericeae and Extended-Spectrum Beta-Lactamase (ESBL)-producing Vancomycin-Resistant Enterococci (VRE). The increasing resistance of certain bacterial pathogens to the clinically potent antimicrobial agents is one of the major challenges that affect health care system worldwide. In the year 2019, the Center for Disease Control and Prevention (CDC) reported more than 2.8 million antibiotic-resistant infections in the United States each year, and more than 35,000 related deaths. From this report, CDC included in their urgent threat list the Carbapenem-Resistant Acinetobacter, Carbapenem-Resistant Enterobacteriaceae (CRE), and *C. difficile* as the most common cause of hospital associated infections. Carbapenem-resistant Acinetobacter are common in ICU patients and can cause pneumonia, as well as wound, bloodstream, and urinary tract infections (Ayobani *et al.,* 2019). The control of the spread of such infections is challenging because it frequently contaminates healthcare facilities' surfaces and shared medical equipment, causing outbreaks in these facilities (Ayobani *et al.,* 2019). The CRE is one of the major concerns for patients in healthcare facilities, principally among patients requiring invasive devices (e.g. catheters) or who have long antibiotic therapy, because some bacteria of this family are resistant to nearly all antibiotics available (Brink *et al.,* 2019). *Clostridium difficile* infections can cause life threatening diarrhea and are often acquired after antibiotic treatment for other medical conditions, with the most serious infections being developed after the use of fluoroquinolones (Sliming *et al.,* 2020). These infections are severe and most commonly obtainable in elderly patients due to their compromising immunity.

Antimicrobial resistance even to single antibiotic may pose a serious problem to hospitalized and out-patients, this is because the use of second- and third-line treatments can have serious adverse effects for the patients and increase recovery time, sometimes for months or even years. Hence, preventive therapy measures to these infections and antibiotic stewardship are the most important strategy in healthcare facilities to reduce the transmission and spread of antibiotic-resistant bacterial pathogens.

Causes of Resistance to Antimicrobial Agents by Nosocomial Bacterial Pathogen

Antimicrobials are commonly used in health care facilities for the treatment of hospital associated infections, community acquired infections and also for surgical prophylaxis. However, several studies have shown that this use is commonly performed using incorrect antimicrobial selection, dose, route of administration, and even the duration of the treatment (Spirak *et al*., 2018). This inappropriate use of antibiotics, especially the broad-spectrum, leads to the emergence and spread of antimicrobial-resistant (AMR) bacteria (Holmens *et al.,* 2018). Another problem with misuse of antibiotic is its associated side effects such as allergy and toxicity that affects normal systems function which could lead to disorder of the human gastrointestinal tract microbiome, with the patient being at risk of developing gut infections by *C. difficile*.

Currently, the Corona virus disease 2019 (COVID-19) pandemic is boosting the use of antibiotics that could lead to the increase in AMR bacteria, because COVID-19-hospitalized

patients often receive empiric broad-spectrum antibiotic therapy (COVID 19 2020). Besides that, hospitalization increases the risk of acquiring HAIs and also contributes to the increased use of antimicrobials (COVID 19 2020). Another problem is the prioritized allocation of isolation rooms to COVID-19 patients, causing cohorting and/or management in open bays of patients colonized with VRE, MRSA, CPE, or *C. difficile*, and the higher workload of healthcare workers can lead to a greater number of hospital transmissions (Rawson *et al.*, 2020).

The main cellular targets of the currently available antibiotics are the cell wall synthesis, protein synthesis, RNA polymerase, DNA replication, and folic acid metabolism (Rawson *et al.*, 2020).

The cell wall synthesis inhibition functions through disruption of the structure of the bacterial cell wall resulting in the leakage of important solutes component crucial for normal cell functions, leading to death of the cell. The β-lactam antibiotics, including penicillin, cephamycins, cephalosporins and carbapenems, target the cell wall biosynthesis in bacteria. In addition, antibiotics such as bacitracin, vancomycin and daptomycin also interact with bacterial cell wall and affect its biosynthesis processes. Another mechanism of action is the inhibition of protein synthesis via irreversible binding to the 30S or 50S subunits of the ribosome (Rawson *et al.*, 2020). This ribosomal attachment results in the inhibition of protein synthesis mechanisms. As protein remain the building blocks of several cellular structures, disruption in their synthesis block the normal bacterial metabolism, inhibits bacterial growth and led to death. The classes of antibiotic include tetracyclines, macrolides, aminoglycosides and streptogramins are good protein synthesis inhibitors. Another class of antibiotics like quinolones and rifampin act by inhibiting the nucleic acid synthesis through bonding to crucial components involved in DNA and RNA synthesis. These two essential cell components are fundamental for all metabolic processes; therefore, the disruption of the synthesis of nucleic acids will affect bacterial multiplication and survival. Antibiotics can also be classified as anti metabolites, including sulfonamides and trimethoprim, which are responsible for the inhibition of other metabolic processes and act on selected cellular processes decisive for the survival of microorganisms (Rawson *et al.*, 2020).

Development of Antimicrobial Alternatives for the Treatment of Nosocomial Infections

The development and marketing strategies of new chemotherapeutics is far behind the increasing emergence of drug-resistant bacteria. Most of the pharmaceutical companies face a lot of challenges in the search of new antibiotics due to identification of number of new cases of bacterial resistances. In fact, only one out of five infectious disease drugs that have started testing in humans were expected to receive approval from the United State Food and Drug Administration (FDA). To address these challenges, several researchers globally, have embarked on the development of potent antimicrobials based on molecules with new modes of action or unique interacting targets from those previously known. The ideal antibacterial agent should also be nontoxic to the host and should have exceptional blood/fluid circulation, as well as absorption, distribution, metabolism, and excretion (ADME) properties, allowing a large therapeutic window with a low dose (Blair, *et al.*, 2015). However, development and marketing of new antimicrobial agents to curtail the emergence of resistance in nosocomial bacteria is highly expensive and involve a lengthy process. The scarcity of commercially produced antimicrobial compounds in the markets makes it a call for the researchers to develop a novel compounds and formulations to tackle the emergence of multidrug resistance. Some of the approaches have been adopted to combat nosocomial infections, which include repurposing the existing drugs, development of metal nano particles, and metal

complexes. The microbial metabolism disrupting compounds such as iron and zinc chelators, antimicrobial peptides and antisense antimicrobial therapeutics are also considered as the potential compounds for the treatment of microbial infections including nosocomial. In this review, we focused on development of modified of drugs, novel metal-based complexes, antimicrobial peptides, and antisense antimicrobial therapeutics (Blair *et al.,* 2015).

Modification of Existing Drugs

Modification of existing drugs is used currently as a strategy in providing new therapeutic compounds able to curtail the threats of multi-drug resistance. It consists of finding a new applicability to existing drugs, rather than their primary medical indication (Ashburn *et al.,* 2004). This approach has become especially important in an industry where the output has not been compensating the spending in the pharmaceutical resources development and the pressure imposed by the high prices, generics' competition, and regulatory issues, altogether inflicting a hard challenge for the discovery of new drugs (Singh *et al.,* 2019).

Drug modification is rooted in two prepositions: drugs that act on various targets and diseases that share the same biological targets (Jourdan, *et al.,* 2020). Candidate drugs to be modified must be under clinical development and safety evaluation, even though they failed to show efficacy in late clinical trials. Moreover, the ones that had their project interrupted due to commercial issues are under exploitation in new geographical markets or, despite being already in the market, have generic or close to expiring patents, and thus can also be assigned to new medical indications (Frie, *et al.,* 2020).

Drug modification strategy offers several merits over development and discovery of new therapeutics. Indeed, there is a significant reduction of the processing time and development risks and costs, since repositioning candidates' pharmacokinetic and safety profiles are already determined due to the stages of clinical development they went through (Ashburn, *et al.,* 2019). In some cases, they were also submitted to other development steps, such as in vitro and in vivo screening, chemical optimization, and toxicology, which are steps that can therefore be skipped (Ashburn *et al.,* 2019). Multiple drugs used and currently in use to treat pathological conditions caused by multi-drug resistant (MDR) pathogens have demonstrated *in vitro* and *in vivo* broad-spectrum of antimicrobial activity. These drugs are normally called "nonantibiotics" and can express antibacterial properties by having direct antimicrobial activity (antimicrobial nonantibiotics), enhancing the effectiveness of an antibiotic when co-administered (synergism; helper compounds), or impairing the microorganisms pathogenicity and activity, similar to modulating the activity of macrophages (Frie, *et al.,* 2020).

The Use of Metal-Based Complexes in the Development of Therapeutics

The use of metal-based complexes has become a major pillar in medicinal chemistry after the approval of platinum in chemotherapy. Over the last two decades, metal complexes of titanium, iron, ruthenium, gallium, palladium, silver, gold, bismuth, and copper have reached clinical trials for cancer, malaria, and neurodegenerative disease treatment (Evans *et al.,* 2021). The interest in metal-based complexes as alternative antimicrobials is also rising due to their potentials in performing ligand exchange and depleting essential substrates, which is not accessible to organic compounds. The shape of a molecule is one of the determining factors in the antimicrobial susceptibility. Metal-based complexes can display multiple geometries and possess a more defining dimensional arrangement in contrast to organic molecules. Hence, the structural properties of these molecules can be related to higher clinical activities. Lately, this research has been extended to their potential as antimicrobials, and these novel potential

compounds were extensively reviewed in which the mechanism of action of silver and gold-based complexes is further assessed (Claudel *et al.,* 2020 and Evans *et al.,* 2021).

The antibacterial activity of silver ions was first described in the 19th century, and colloidal silver was accepted by the FDA as being effective for wound management in the 1920s (Klasen *et al.,* 2021). However, the emergence of penicillin as a novel antibiotic in the year1940, the use of silver-based complex was diminished. In 1968, silver nitrate was combined with sulfonamide to form silver sulfadiazine, and was started to be used as a broad-spectrum antibacterial cream for the treatment of burns (Fox *et al.,* 2020). Silver sulfadiazine was found to have activity against *Staphylococcus aureus, Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeriginosa*. Silver but not sulfaziadine was shown to binding affinity to bacteria pathogens. The potency as well the efficacy of silver sulfadiazine complex is thought to emerge from its slow and steady reactions with serum electrolytes and other sodium chloride (NaCl) contained in the body fluids. This allows the slow and sustained delivery of silver ions into the wound micro environment. Recently, researchers began the use of wound dressings to incorporate silver as alternative treatment diseases caused by multi-drug resistant bacteria such as Methicinin-Resistant *Staphylococcus aureus* (MRSA). First-line antibacterial drugs have shown clinical limitations in the last 20 years and several researches have also shown the antibacterial activity of silver-based complexes against Gram-positive and Gram-negative bacteria (Nunes *et al.,* 2016). Some of the new metal-based complexes that reveal strong antimicrobial activity against resistant respiratory bacterial pathogens, including *B. cepacia* complex strains are N-heterocyclic carbene (NHC) complexes of silver (I). Camphor-based silver complexes have also shown promising antimicrobial properties against bacterial pathogens, being more active against Gram-negative bacteria, including *P. aeruginosa* and Burkholderia contaminants (Carvallo, *et al.,* 2019, and Costa *et al.,* 2021).

Metal-Based Nanoparticles

Over the last ten years, there has been a constant emphasis on creating novel approaches and treatment breakthroughs to manage Multi-drug Resistant bacterial strains in the post-antibiotic period. Nanotechnology has been discussed as a potential solution in this regard. (Ananda *et al*., 2022). Recent advancements in nanotechnology have led to the development of nanomaterials, particularly metal nanoparticles (NPs) with antimicrobial activity. These NPs have demonstrated significant potentials as an alternative to conventional antimicrobial treatments (Cheeseman *et al.,* 2020). A nanomaterial refers to any substance that has been reduced in composition to the nanoscale, typically within the range of 1–100 nanometers, or possesses at least one dimension in the three-dimensional space at the nanoscale (Wu *et al.,* 2020). The two main ways used for the synthesis of nanoparticles (NPs) includes; the top-down and the bottom-up approaches. Additionally, there are three strategies employed for NP synthesis: physical, chemical, and biological methods (Dikshit *et al*., 2021). The unique properties of NPs, including micro size, higher reactivity, and large surface areas compared to their large counterparts, along with the tenability of their features, have significantly contributed to the advancement of nanoscience. These characteristics have accelerated the application of NPs across various fields, such as biomedicine and other scientific disciplines (Salem *et al.,* 2022). Moreover, a few parameters, which primarily impact the bactericidal and biocompatible qualities of metal NPs, are essential for the antibacterial activity of these particles. These parameters include small size, shape, surface area to volume ratio and surface functionality. In this situation, designing NPs with the right physico-chemical characteristics might minimize their harmful effects and reduce the dangers associated with using them in biomedical applications (Franco *et al.,* 2022). Nanoparticles demonstrate a significantly enhanced capacity for physical interaction with bacterial cells compared to some antimicrobial

agents. This is primarily attributed to their smaller size and higher surface area-to-volume ratio (Ananda *et al.*, 2022). The interaction between bacterial cells and metal ions occurs through electrostatic forces, where the negatively charged surface of the bacterial cell membrane easily adheres to the positively charged metal ions produced by NPs (Shilpa *et al.*, 2022). This leads to the generation of free radicals through the activation of free radicals through the activation of reactive oxygen species (ROS), which in turn causes a disruption in the cell membrane, the deactivation of important enzymes and the destruction of microbial nucleic acids (DNA and RNA). Ultimately, these processes culminate in the cell death of harmful bacteria (Hasani *et al.*, 2019).

The first study to compare the antibacterial efficacy of various metal ions and CTSE-synthesized NPs against nosocomial infections in burnt wound intensive care units (BWICU) and intensive care units (ICU) was conducted by Ramteke *et al.*, (2020). This research reviewed two metal ions, four monometallic nanoparticles and two alloy noble metal nanoparticles for their minimum inhibitory concentration (MIC) against nosocomial pathogens. The findings revealed that, silver NPs synthesized from $AgNO_3$ and Ag_2SO_4 has cytotoxic activity against all tested isolates. Furthermore, silver-gold alloy NPs synthesized from silver nitrate and gold salts demonstrated higher efficacy and potency compared to those made from silver sulfate and gold salts. However, only *Staphylococcus aureus* and *Escherichia coli* demonstrated susceptibility to the effects of gold and copper NPs, respectively. Additionally, when compared to copper ions, monometallic and alloy NPs exhibited lower cytotoxicity than ionic silver.

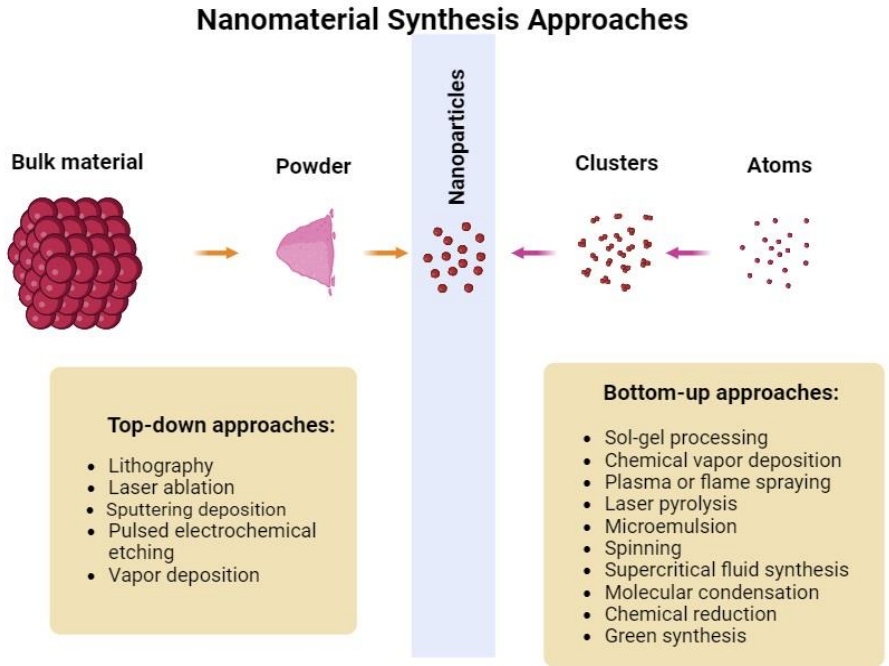

Fig 1. Schematic representation of NPs synthesis approaches.

Antimicrobial Peptides (AMPs)

Antimicrobial peptides (AMPs), a diverse group of bioactive small proteins, are essential host defense components that are ribosomally synthesized by most life forms, including bacteria, archaea, fungi, plants, and animals (Wuerth, *et al.*, 2019). Natural AMPs are usually rich in positively charged amino acid residues (such as lysine, arginine, and histidine), have an amphipathic nature, and show a broad spectrum of activity against a wide range of pathogens (bacteria, fungi, parasites and virus) (Beisswenger, *et al.*, 2017). Structurally, these

antimicrobial peptides are generally categorized into: β-sheet, linear α-helical, extended, and cyclic peptides, and their physicochemical parameters influence the mechanism action of antimicrobial peptides (AMPs). The stereotypical mechanism of AMP action is to integrate into the bacterial cell membrane and disrupt its integrity, leading directly or indirectly to bacterial cell lysis and death (Berlund, *et al.,* 2015). Although bacterial killing by AMPs commonly occurs via membrane perturbation mechanisms, they can also exhibit more complex activities, including bacterial cell penetration and interference with vital intracellular processes (e.g., metabolic and translation inhibition) (Gaynon, *et al.,* 2016, Florin *et al.,* 2017, and Hale *et al.,* 2014).

In addition to the fast killing kinetics, pharmacodynamic properties, and mechanisms of killing that overcome common resistance mechanisms of MDR pathogens, AMPs may elicit an anti-infective host immune response and possess the ability to neutralize toxins (Lazzaro, *et al.,* 2020, Friedrich *et al.,* 2015, and Lin *et al.,* 2018). The anti biofilm properties of these molecules may also confer efficacy against infections associated with wounds, medical implants, and chronic respiratory illnesses (Melvin *et al.,* 2016, and Lashua, *et al.,* 2016).
Considering the devastating nature of antimicrobial resistance, several attempts have been made to search for Antimicrobial Peptide (AMPs) based effective chemotherapeutics. Up till now, there are several clinically relevant AMPs that were reported to show antimicrobial, anti-inflammatory, anti biofilm, and wound healing activities. Nevertheless, only a few of these peptides have proceeded to preclinical studies or clinical trials, obtained Food and Drug Administration (FDA) approval, or have been launched on the market.

Antisense Antimicrobial Therapeutics
Antisense RNAs (asRNAs) are ubiquitous in bacteria and are involved in a wide range of functions, from central metabolism to pathogenesis-related mechanisms (Brophy, *et al.,* 2016, and Pita, *et al.,* 2020). This strategy can be turned to our favor by using synthetic RNAs to fight pathogens, targeting metabolism and/or antibiotic resistance genes (Miller *et al.,* 2021). Antisense is among the best emerging alternative therapy over clinical antimicrobial targeted to a greater extent reduce the duration of new antimicrobial discovery, development and enable treatment specific to a target gene or entire pathogen. However, these therapies are still far from being a common antimicrobial approach, mostly due to the challenge of delivering oligomers to bacterial cells (Sully *et al.,* 2016).

The mechanism of action of Antisense oligomers (ASOs) is by binding to the target mRNAs with a complementary sequence. This interaction inhibits the mRNA translation into protein through steric blockage and/or through RNase degradation of the ASO/RNA duplex (Robert *et al.,* 2020). To make Antisense therapy clinically possible, the Antisense oligomers chemical structure is to be modified. The modification of ASOs sugar, backbone, nucleobase, and 3′- and 5′-terminal can improve their stability, avoid nucleases attack, and preserve target specificity (Janis *et al.,* 2021). Antisense oligomers (ASOs) modifications are mainly four: phosphorothioates (PS) linkages, locked (bridged) nucleic acids (LNA/BNA), peptide nucleic acids (PNA), and phosphorodiamidatemorpholino oligomers (PMO) (Sully *et al.,* 2016). Antisense oligomers (ASOs) modifications have been recently reviewed by Hegarty and Stewart (Hegety, *et al.,* 2015). To solve the problem of delivery-free antisense oligomers (ASO) through the cell wall of the bacterial pathogens, researchers are currently developing some strategies using carriers such as Cell-penetrating peptides (CPPs) and diverse nano materials which can effectively and efficiently transport ASO across the bacterial cell wall. They also developed a PMO conjugated with CPP to target a highly conserved region in the *Escherichia coli* gyrA gene sequence (Ashburn *et al.,* 2019). Study have shown that targeting gyrA with a

CPP–PNA reduces the viability of some pathogens such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Streptococcus pneumoniae*, *Enterococcus faecalis* as well as *Streptococcus pyogenes*. The study also revealed that, combination of CPP (bacteriostatic) and PMO (bactericidal) has leads to higher antimicrobial activity. In addition, targeting the *S. pyogenes* gyrA can have a synergistic effect when applied alongside levofloxacin, novobiocin, or spectinomycin (Potenge *et al.*, 2017). Barkowsky *et al.* 2019 tested several CPPs and observed that HIV-1 TAT, Oligolysine (K8), and (RXR) 4XB were the most efficient to abolish bacterial growth *in vitro* (Barkowsky *et al.*, 2019).

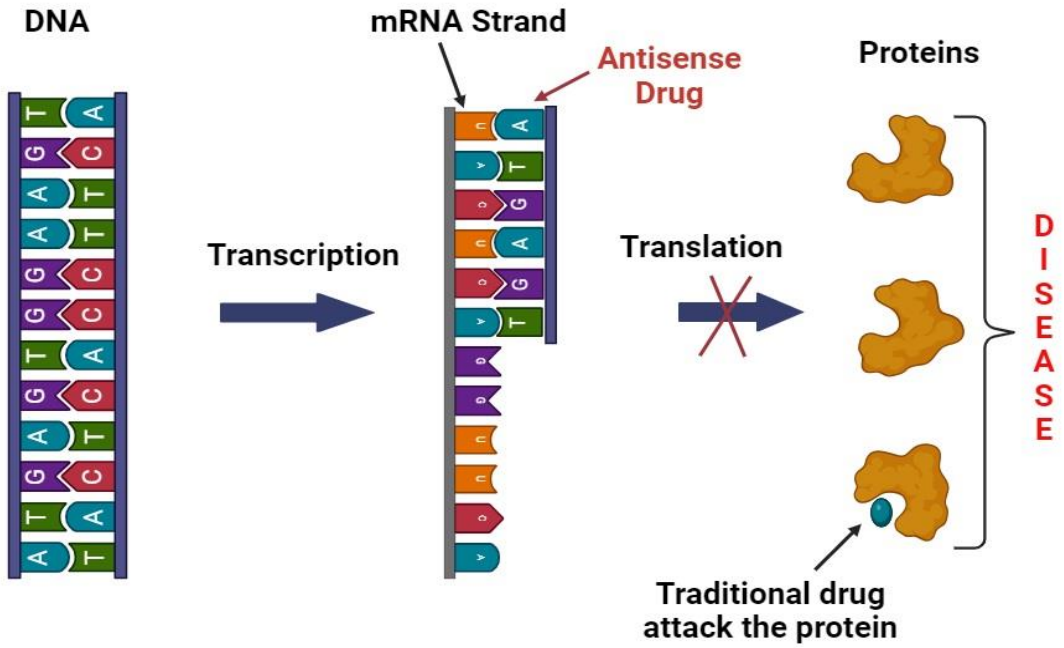

Fig 2. Schematic representation of antisense therapeautic mechanisms.

CONCLUSION

Emergence of antimicrobial resistance to nosocomial bacterial pathogens is a world-wide threat to public health systems by reducing potency and efficacy of antimicrobials against the resistant bacteria. It also poses an increased associated risk of performing invasive and noninvasive medical therapies and procedures that rely on antibiotics administration to reduce complications.

To come up with lasting solution to this global medically challenging issue, search of reservoirs of existing nanoparticles with antibiotic activity is currently in the pipeline, and is leading to the emergence of new antibacterial with enhanced advantage of decreasing the necessary time for the antibacterial drugs discovery, development, approval and commercialization. However, drug modification will not solve all nosocomial bacterial infections and drug resistance-associated problems. Therefore, novel compounds such as metal-based complexes and Antisense oligomers with antimicrobial properties and with unique ability to target the gene of the pathogen are also required to address the challenges of multi-drugs resistant infections caused by the nosocomial bacteria. Efforts by several researchers worldwide to discover and develop potent antimicrobials to deal with multi-drug resistance in pathogens using verity of molecules and approaches are in use. These efforts are

anticipated to lead in the near future, the introduction of new antimicrobials into the markets to combat nosocomial infections.

Nevertheless, the target sites as well as the mechanism of activity of these new compounds are still under investigation. Hence, Omics technologies could be a recommended approach for the identification of their drug targets and the bacterial mechanisms of resistance.

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