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Review Article
Role of Nanocarrier Systems in Drug Delivery for Overcoming Multi-Drug Resistance in Bacteria

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Abstract

Multidrug-resistant (MDR) bacteria have risen alarmingly in the last few decades, posing a serious threat to human health. The need for effective bacterial resistance treatment is urgent and unmet due to the rise in morbidity and mortality that has coincided with the prevalence of infections caused by MDR bacteria. Using its creative and unconventional methods, effective antibiotics for MDR bacteria could be developed using nanomedicine techniques. To combat microbial resistance, a number of strategies have been developed, including the use of natural bactericides, the introduction of fresh antibiotics, the application of combination therapy and the creation of NP-based antibiotic nanocarriers. The absence of novel antibacterial agents has worsened the situation for MDR bacteria. Ineffective antibiotics used to treat MDR bacteria also contribute to the bacteria's tolerance growing. Nanoparticles (NPs) are the most efficient method for eliminating MDR bacteria because they serve as both carriers of natural antibiotics and antimicrobials and active agents against bacteria. Additionally, surface engineering of nanocarriers has important benefits for focusing on and modifying a variety of resistance mechanisms. The use of nanocarrier systems in drug delivery for overcoming bacterial resistance is covered in this review along with various mechanisms of antibiotic resistance.

Key words: Nanomedicine, nanocarrier, antibiotics, multi-drug resistance, intrinsic resistance, acquired resistance

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INTRODUCTION

Multidrug resistance (MDR) is a type of insensitivity that microorganisms can develop to antibiotics at lethal doses. Regarding antibiotics' effectiveness against pathogenic diseases, MDR has grown to be a major concern[1]. According to statistics on infections brought on by MDR bacteria, the sensitivity of bacteria to antibiotics has multiplied significantly recently. According to a number of reports, antimicrobial resistance in Europe poses a serious threat to people's health[2]. According to reports by Yang *et al.*[3], dealing with the mortality rate caused by MDR bacterial infections would cost Europe's economy €1.5 billion annually.

The MDR bacterial infections, on the other hand, affect over two million Americans annually and result in the deaths of about 23,000 people. The additional societal and healthcare costs associated with these diseases total €1.5 billion annually in the United States[4]. The situation of MDR bacteria has gotten worse due to the lack of new antibacterial agents being developed[5]. The use of ineffective antibiotics to treat MDR bacteria also contributes to the expansion of the bacteria's tolerance. Methicillin resistance affects 40-60% of *Staphylococcus aureus* strains gathered from various US hospitals and in some cases, resistance to vancomycin and carbapenems is also present[4,6].

In addition to decreasing the effectiveness of antibiotics in treating infectious diseases that are life-threatening, antibiotic resistance in bacteria raises the overall cost of therapeutic approaches. One of the difficult tasks for biomedical scientists has been overcoming bacterial antibiotic resistance. This review focused on the mechanisms by which MDR in bacteria develops as well as the most recent developments in the use of nanocarrier-based antibiotics to treat MDR in bacteria.

Health problems and the economic impact of MDR: The effectiveness of antimicrobial agents is significantly impacted by microbial agent resistance, which is linked to high mortality rates and high medical costs (Fig. 1). By raising the possibility of the spread of resistant pathogens, impairing treatment effectiveness and lengthening the time it takes for patients to become infected, MDR poses a barrier to the management of disease[7]. The quality of public hygiene and the variety of bacterial and fungal pathogens' resistance profiles have a big impact on how effective antimicrobial agents are. Treatment costs rose as a result of pathogen resistance to already available drugs, necessitating more expensive therapies.

The MDR has also been significantly aided by the effective current use of medical procedures like organ transplantation and cancer chemotherapy. A number of products that disrupt the financial systems of developing nations are less likely to be imported and exported as a result of increased tourism and international trade[8]. High-potential MDR pathogens result from this and spread globally.

Multidrug resistance in bacteria: cellular and molecular mechanisms: Understanding microbial genetics and the process of genetic modification will help in the fight against resistance mechanisms on a number of levels[9]. According to Saha and Sarkar[10], Cox and Wright[11], resistance mechanisms can be divided into acquired, integral and intrinsic types.

FIRST, INTRINSIC RESISTANCE

A molecular phenomenon known as intrinsic resistance is based on the inherent or integral traits that bacteria have developed over time to resist antimicrobial agents. Due to a lack of antibiotic-based selective stress, microbes' natural resistance trait occasionally experiences natural genomic changes[11]. Although generally speaking, pathogen adaptation is sparked by antimicrobial microecological stress. A drug-resistance gene can be acquired through evolutionary competition or mutations and this can happen as a result of several distinct events, as shown in Fig. 2 and described as follow.

Change or lack of target site: Microbes must assimilate antimicrobial compounds to take targeted action. Antimicrobial agents can pass through the bacterial cell membrane with the help of beta-barrel proteins (porins) present in the microbial membrane. Some bacteria can regulate their outer membrane to fend off outside antimicrobials. For example, some Gram-ve bacteria may alter the selectivity, abundance and size of membrane porins in order to reduce the uptake of certain antimicrobials such as aminoglycosides. A similar insensitivity to antibiotics with a β-lactam moiety is caused by mutations in penicillin-binding protein (PBP) sites[13].

Target site' species-specific structure: It is almost immediately apparent that antimicrobials act in essentially the same ways in different bacterial communities. However, in some instances, species specificity contributed to antibiotics' low affinity for their target site. Even bacteria from the same genus can alter the antibiotic binding site by developing resistance to the antibiotic and taking on different structural forms for the same target. For instance, the large ribosomal subunit of *S. aureus* has distinct binding modes and structural forms for various antibiotics, according to Antshel *et al.*[14].

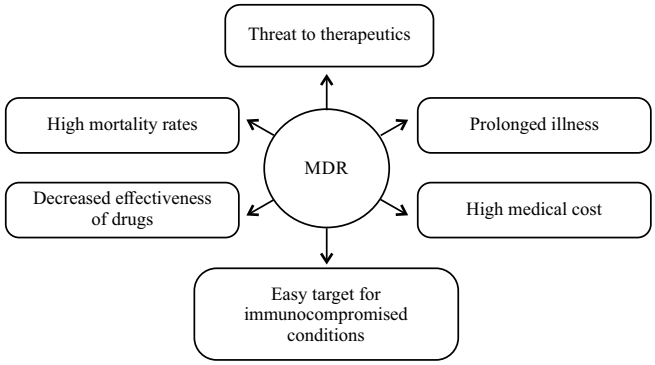

Fig. 1: Numerous issues with multidrug-resistant bacteria in terms of society and health[8]

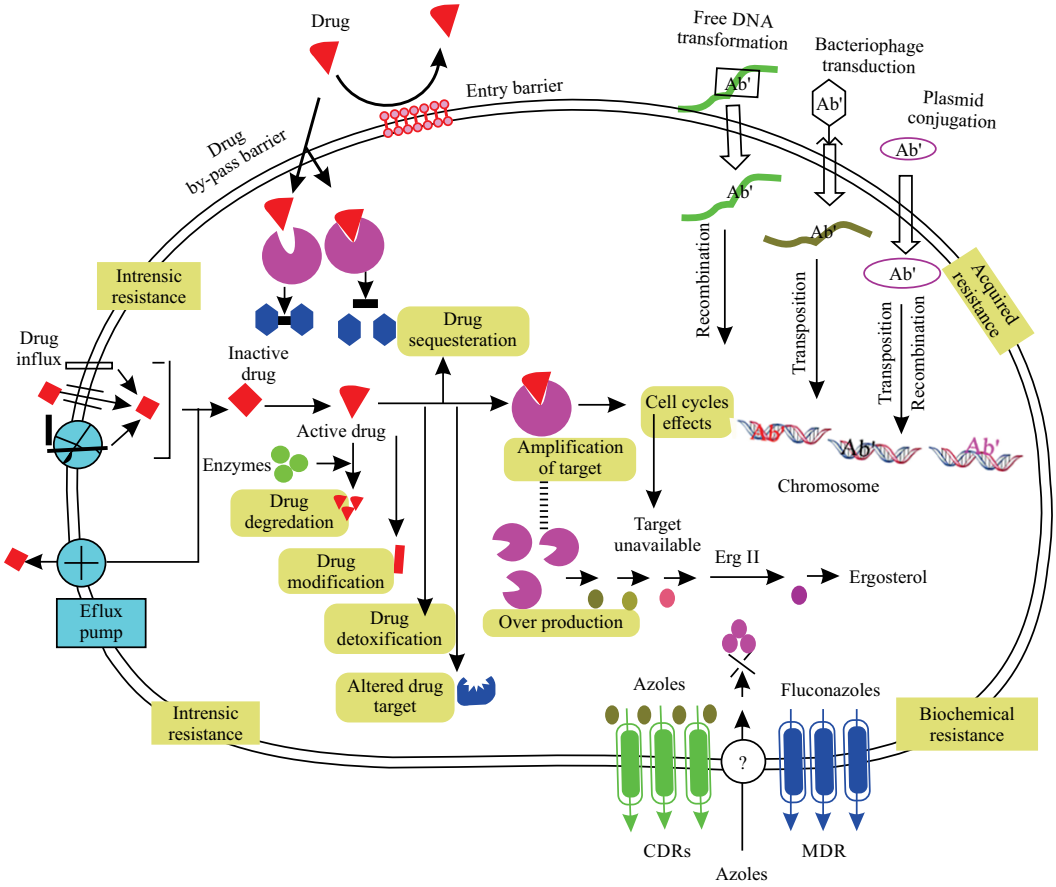

Fig. 2: A schematic representation of various molecular mechanisms for microbial resistance[12]

Inactive antimicrobial agents: The active ingredient of the therapeutic agent is one of the efficient strategies used by microbes to protect themselves. For instance, a -lactamase enzyme hydrolyzes the active-lactam ring in cephalosporins and penicillins to produce inactive penicilloic acid. As a result, antibiotics are unable to bind to PBPs, protecting the bacterial cell membrane from harm[15]. According to research by Bockstael and van Aerschot[16], it has been discovered that this technique of inactivation against aminoglycosides, chloramphenicol, etc., is used by both Gram-positive and Gram-negative bacteria.

Availability of efflux pumps: In order to have a long-lasting impact, an antimicrobial agent needs to be present in a microbial system for a long time and in high concentrations. Some bacteria, though, have incredibly powerful drug pumps that force the medication out, leaving insufficient medication for it to work as intended. Some MDR pumps even selectively displace particular antibiotics, including tetracyclines, streptogramins, lincosamides and macrolides[17], despite the fact that MDR pumps extrude a variety of functionally and structurally distinct medications. Except for ATP-binding cassette (ABC) transporters, proteins of all efflux families are driven to engage in efflux activities by sodium and proton. In contrast, primary ABC transporters engage in efflux activity in response to ATP hydrolysis. *Streptococcus pneumoniae* and *S. aureus* have been found to use these strategies.

Neutralising capacity that is high: Some bacteria produce poisonous substances as a defense against rivals and potential predators. They also want to escape the damaging effects of the toxic chemicals they release at the same time[18]. This pertains to *Streptomyces* spp. and other bacteria that are used to produce antibiotics. By using acetyltransferases and phosphotransferases, these bacteria can render their own antibiotics, like neomycin and streptomycin, inactive. The erythromycin-producing bacterium *Saccharopolyspora erythraea* (previously *Streptomyces erythraeus*) methylated the target site, the H rRNA, in order to defend itself[19].

Little drug concentration: Drug instability, low bioavailability, rapid metabolism and short circulation times all contribute to insufficient drug concentration in the host system. The low drug concentration at the focal site may be caused by all of these factors, but as the drug is exposed to the biological environment, the microbes or cancer cells may develop resistance[20].

Response to stress: The genetic mutation of a cell can also be influenced by other environmental factors like oxidative stress, oxygen deprivation, viral infection, trauma, heat, UV radiation, osmotic stocking, and pH. These mutations offer resistance to stressors and antimicrobial agents. The oxyR network, the heat shock response, the response to alkylating agents and the SOS response are the four stress-related regulatory systems that are typically present in prokaryotes. For instance, the heat shock proteins dnak and groEL can be produced by *E. coli* in response to UV radiation, hyperthermia and even nalidixic acid[21]. Asserts that the ability of *Salmonella typhimurium* to adapt to H_2O_2-induced oxidative stress also confers resistance to heat killing.

IMPLEMENTED RESISTANCE

The process of gene exchange/transfer or gene mutation through the processes of conjugation, transduction, or transformation are part of the acquired resistance mechanism, According to Milinevsky *et al.*[22] and Flintoff[23], after transfer of resistance genes, biomechanical activity or overexpression of the genes alters the drugs in a way that renders them ineffective. For instance, the regulation of SOS signals is impacted when the LexA repressor gene in *E. coli* is mutated[24]. Aside from, when they are resistant to H_2O_2, typhimurium alters the expression of a number of stress-regulating genes, including glutathione peroxidase, SOD, catalase etc.

Genetic alteration based on chromosomal changes: One of the most adaptive ways for microbes to acquire resistance to drugs is a change in drug targets. According to Hooper and Jacoby[25], the efflux pump mechanism and genetic changes are responsible for fluoroquinolone resistance. Fluoroquinolones gain resistance when drug targets like DNA gyrase and topoisomerase IV are altered. Since each target consists of two subunits (GyrA and GyrB for DNA gyrase and GrlA/ParC and GrlB/ParE for topoisomerase IV), their role in DNA duplication is crucial. The function of one of these targets is ATP binding and hydrolysis, whereas the function of the other target is DNA binding. Antibiotic resistance is brought on by mutations in the DNA binding domain region, which controls multiple mutations introduce additive effects to increase the resistance characteristics of the bacteria.

Similar to how the RpoB point mutation affects drug binding affinity on the RpoB subunit, rifamycins can be used as a first-line therapeutic agent for tuberculosis infection alone or in combination with streptomycin, isoniazid and other drugs. The drug's dihydropteroate synthase enzyme activity is decreased as a result of the sulfonamides it targets for modification. Trimethoprim blockade causes dihydrofolate reductase to mutate, which causes excessive protein induction and decreased drug affinities. Point mutations on the 23S rRNA and 16S rRNA operons confer resistance to the antibiotics macrolide, lincosamide and streptogramin (MLS) as well as tetracycline[26].

Genomic replacement: The majority of gene mutations are related to gene overexpression or gene amplification events. To confer drug resistance, genomic duplication is a relatively common process in eukaryotic cells. Since many transporters are overexpressed as a result of this genetic induction, the biosynthetic machinery is improved. Tetracycline exposure causes the acrAB locus in *E. coli* to be amplified genomically,

which facilitates the acrAB efflux pump systems that result in the MDR phenotype[27]. *Staphylococcus aureus* has also been linked to a similar duplication process that results in methicillin resistance. One defense mechanism against the constraints of mutational aspects is genome amplification. However, it is well known that without medication, microbes return to their normal state.

Aim specific for modulated drugs: The first strain of bacteria resistant to methicillin and penicillin was a-lactamase-producing organism called *Staphylococcus aureus*. The resistance mechanism responsible for altering PBPs through genetic mutations that give *Staphylococcus* and *Streptococcus resistance* to-lactams. In resistant *S. aureus*, mec A encodes this gene on a motile gene unit[28]. According to drug exposure, the transglycosylase and transpeptidase activities of resistant *S. aureus*' PBP2 enzyme change, indicating the bacteria's tolerance or sensitivity to a given drug[27].

The majority of plasmid-transferred Qnr elements are found in gram-ve non-typhoidal *Salmonella*, *Shigella*, *E. coli* and other species that are highly sensitive to fluoroquinolones[27] pentapeptide repeating proteins MfpA and Qnr defend DNA gyrase and topoisomerase II, respectively, which controls fluoroquinolone resistance. The Qnr also protects topoisomerase from the negative effects of drugs. Additionally, MfpA interacts with DNA gyrase in *Mycobacterium* and results in structural features and activity that are comparable to those of the B-DNA inhibitor ciprofloxacin[29]. By combining with other modes, MfpA and Qnr can enhance the resistance profile to sophisticated levels.

Permeability channel of membrane and Efflux mechanisms: Resistance is typically brought on by the drug's weak or minimal interaction with its cellular targets as a result of drug efflux, as opposed to being restricted to drug internalization and uptake. The drug was first discovered in the emergence of tetracycline resistance[27,30]. The medication is expelled from the cell by an efflux pump mechanism. The ABC group of primary transporters, which drives the efflux mechanism, also participates in drug ejection via a proton-driven gradient force along with the remaining side groups[31,32].

Proteins that are involved in drug excretion can be divided into systems with one component that carry a specific substrate domain or systems with two components that allow the binding of various structural compounds and produce a variety of resistance phenotypes. Cytosolic proteins can cross both the outer and inner membrane barriers thanks to transporters for resistance nodulation cell division (RND). The transcriptional repressor has space to regulate protein expression thanks to the transmembrane regions of tetracycline efflux transporters. By using medication to disable the repressor, the tetracycline efflux machinery is encouraged to express.

Surface-engineered nano-cargos applications for controlling antibiotic resistance: Due to their dual functionality as carriers of natural antimicrobial agents and antibiotics as well as active agents against bacteria, nanoparticles (NPs) offer the most effective method of eradicating MDR bacteria[33]. Whether contained within the structure or affixed to its surface, NP-based DDS can deliver a variety of therapeutics to the infection site in a safe and efficient manner[34]. Additionally, nanoparticles are candidates for better therapeutic efficacy against MDR bacteria due to their distinct physicochemical characteristics[6]. It is challenging for bacteria to develop resistance to nanoparticles because they can act through a variety of bactericidal pathways[35].

The NPs' capacity to kill bacteria is influenced by their size, shape, basic core material and surface chemistry. Furthermore, NPs stand out for antibiotic delivery due to their high antibiotic load and high biological membrane penetrability. Additionally, the effectiveness of nanocarriers and treatments can be significantly increased by altering how nanoparticles interact with bacteria's cell walls or membranes[36].

With the introduction of fresh and inventive approaches to creating powerful antimicrobial agents to combat the problem of microbial infections, nanomedicine has developed into a well-developed area of drug development[37]. Most nanocarriers typically run into at least one of the MDR bacteria's resistance mechanisms. Based on their physicochemical characteristics, which vary for each material, nanocarriers' bactericidal mode of action can be predicted[38]. Unlike conventional antibiotics, the nanoparticles can interact with bacterial cells more effectively due to their nanometer size and larger surface area. These nanocarriers can also successfully get around the bacterial cell envelope, which limits how well antibacterial medications can be absorbed. Metal nanoparticles (NPs), carbon nanotubes (CNTs), dendrimers, cyclodextrin (CD), chitosan (CS), NNO particles (Ps) and antimicrobial peptides (AMP) are a few examples of NPs that have the ability to directly penetrate and break the bacterial envelope. With the introduction of new and innovative methods to create potent antibacterial agents to combat bacterial infections, nanomedicine has become a well-developed field of drug development[38].

Nanotechnology in medicine is of great importance from a therapeutic point of view, but also from a diagnostic point of view. Nanoparticles can be used to precisely deliver drugs to target sites in the body. This helps treat cancer patients with customized treatment plans. Not only can nanoparticles greatly improve drug delivery, but scientists are also working to use nanotechnology to analyze DNA and mechanically reverse plaque buildup in arteries in minutes. They're also working on nanoparticles that deliver insulin to kick-start cell growth in diabetics, preparing tissues for cryopreservation, building new muscles with carbon nanotubes and repairing spinal cord injuries. One of the major limitations in nanoparticle-based drug delivery is clearance through the reticuloendothelial system, 'RES'. It is implied here that size affects both clearance and distribution.

CONCLUSION

It has been determined that the MDR bacteria are a deadly threat to health and are a challenging problem to solve globally. It could monitor and treat infections brought on by MDR bacteria more effectively if we understood how resistance works. The key factors in bacterial resistance to antibiotics include biofilm formation, overexpression of the efflux pump, molecular genetic modification and resistance development via transferable genetic elements. To combat microbial resistance, a number of strategies have been developed, including the use of natural bactericides, the introduction of fresh antibiotics, the application of combination therapy and the creation of NP-based antibiotic nanocarriers. Finally, despite antibiotic treatment, pathogenic microorganisms can persist because they are capable of developing physiological resistance without genetic modification. Extended-spectrum-lactamases, which 1aqw4e4r5678890 are derived from common TEM-lactamases, are one example of this worrying trend, which is the recent selection of mutants of common resistance genes.

SIGNIFICANCE STATEMENT

The emergence of MDR bacteria poses a threat to public health and represents a major challenge to be addressed. Formation of biofilms, overexpression of efflux pumps, genetic alterations at the molecular level and expression of resistance through transferable genetic elements are important effects of bacterial resistance to antibiotics. Several approaches have been taken to eliminate microbial resistance, including the use of natural fungicides, the development of new antibiotics, the use of combination therapies and the development of NP-based antibiotic nanocarriers. Intensive research is being conducted to suppress microbial resistance through the development of nanoparticle systems. Therefore, it can be concluded that the aforementioned methods, such as incorporating natural microbicides into NPs or encapsulating antibiotics alone, can address microbial resistance.

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