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Review Article

Microbiology for Better Health Care

Review on Recent Advancement of Therapeutic Interventions in Combating Multidrug Resistant Bacteria

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Abstract: Antibiotics is a class of drug that plays an eminent role in combating the deadly infections caused by harmful pathogenic organisms. However, irrational use of these antibiotics leads to multidrug resistance in bacterial species which in turn contributes to the morbidity and mortality worldwide, due to which they are referred to as superbugs. Thus, multidrug resistance is known as the resistance of a microorganism to different initially sensitive antibiotics. Though research has led to the development of new antibiotics and its derivatives, the development of new antibiotics is challenged at every step due to the emergence of microbial resistance. The emergence of drug resistance resulted due to the presence of Drug Resistant Plasmid, transposon, antibiotic resistant cassette and further the resistant phenotype can be transferred from resistant to a sensitive microorganism making sensitive organism resistant. Some bacteria acquire the property of being Multi Drug Resistant due to the presence of efflux transporter proteins which can expel antibiotics in a nonspecific manner and overcoming such medical emergencies is the demand of the era. Thus, the lack of novel antibiotics has created the need for certain technological tools to combat this deadly phenomenon. Among them, anti-microbial peptide, anti-virulence compounds, phage therapy, nanotechnology and CRISPR-Cas system are the most commonly used techniques to combat multidrug resistance. The present review highlights different multidrug resistance tools such as the application of Phage therapy, use of nanotechnology, CRISPR-Cas system and antimicrobial peptides together, their working mechanism, utility, efficacy, credibility and relevance along with the modifications needed in the techniques for improvisation.

Keywords: Antibiotic, Multi Drug Resistance, Bacteriophage, Nanotechnology, CRISPR-Cas System.

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1. INTRODUCTION

During the last few decennials, the prevalence of microbial infections has increased dramatically. Perpetual distribution of antimicrobial drugs in treating infections has led to the emergence of resistance among the sundry strains of microorganisms [1]. Multidrug resistance (MDR) is defined as the resistance of a microorganism to multiple drugs at a time[2,3]. Bacteria usually overcome the antimicrobial activity of antibiotics using three related mechanisms namely resistance, persistence and tolerance[4]. Multidrug resistance in bacteria occurs by mechanisms such as presence of drug resistant plasmid which can undergo horizontal transmission from a resistant bacterium to sensitive one rendering it resistant, presence of transposons and antibiotic resistance cassettes are some other contributing factors to the present medical emergency. Bacteria of certain genera exhibit multi drug resistance due to the presence of multidrug efflux pumps which can pump out multiple drugs non-specifically from the cell[5]. Some bacteria are termed as "Persisters" as they are non-growing, metabolically inactive and dormant and found to play a role in recurrent or chronic infections as they can survive both antibiotic interventions and host immune response, as the drug pressure is removed, they can revert back to its wild type conferring antibiotic susceptibility[6]. Resistant phenotype is exhibited by tolerant bacteria and thus, tolerance is defined as the ability to survive in the conditions of transient exposure to high concentrations of antibiotics. The World Health Organization has studied these and concluded that resistant microorganisms such as bacteria, fungi, viruses, and parasites can resist antimicrobial drugs leading to ineffective treatment resulting in sedulousness and spreading of infections. In the year of 2017, two important studies were done related to the evolution of resistance by tolerant bacteria and persisters. Studies revealed that after long exposure of *Mycobacterium tuberculosis* to Rifampicin the persisters basically results from source of de novo resistant mutants whereas generation of increased tolerance results from induced mutation in *Escherichia coli* population after intermittent exposure of Ampicillin. In the year of 2019 a strong correlation between persister and probability of resistance development in the laboratory isolates of *Escherichia coli*. Many antibiotics are tested upon the clinical isolates and all led to a common conclusion strongly suggesting a link between persistence and tolerance to antibiotics and evolution of resistance to these antibiotics (Table 2). Currently many evidences are there which suggests that many bacteria bear the ability to live inside some cells like macrophages and formation of biofilm are associated with persistent infection. Clinical isolates responsible for chronic infections such as *Pseudomonas aeruginosa*, and uro pathogenic *Escherichia coli* exposed to a long-term antibiotic pressure may result in persistent infection with respect to those responsible for acute infection. As discussed previously persisters results from mutation and studies have revealed the presence of hip A mutation in *Escherichia coli* associated with persistent urinary tract infection and importance of hipA7 mutation resulting in mersister formation *in vitro*. It was determined by various research that a bacterium exposed to high concentration of antibiotic results in the development of Persisters while exposing the same microorganism to low concentration of antibiotics results in development of resistance. Thus, the present condition demands a better therapeutic intervention to overcome multi drug resistance[7]. The review aims at describe the alternative approaches to overcome the threat, application of Phage therapy, Nanotechnology and various Nano formulations, CRISPR-Cas system (figure1) and antimicrobial peptides were highlighted with their recent advancements affecting biofilm formation by inhibiting quorum sensing has been elaborated in details. By studying many cases, the World Health Organization has prepared a report that shows varying rates of resistance in different bacterial species (Table 1). Antimicrobial resistance is a threatening phenomenon associated with high mortality and medical costs and has significance in the efficacy of antimicrobial agents. The ecumenical trade and tourism are expanding continuously leading to the incremented potential of MDR to spread worldwide, also the decrease in export and import of sundry products which directly affects the economy of developing countries [5,9,10]. To combat this deadly effect, the government takes initiative to conduct many programs to optimize antimicrobial therapy, minimize treatment-cognate cost, ameliorate clinical outcomes and safety, and minimize or stabilize MDR [11]. Also, for better-combating purposes, many efficient tools have been designed to combat this, including Phage therapy, Nanotechnology, CRISPR-Cas system as described in this review.

1.1 Technological Tools To Combat Multidrug Resistant Bacteria

Following Table 1 and Table 2 gave an insight to various Multi Drug Resistant Bacteria and their mechanism of Resistance.

Table 1. Common MDR bacterial species along with their respective diseases [12-15].

Name of MDR bacterial species	Drugs resistant to	Diseases
Staphylococcus aureus	Methicillin	Skin related diseases cause wound and bloodstream infections.
Streptococcus pneumoniae	Penicillin	Bloodstream infections, infections in the middle ear.
Mycobacterium tuberculosis	Isoniazid, Rifampicin, and Fluoroquinolone	Pneumonia, urinary tract infections, meningitis.
Klebsiella pneumoniae	Carbapenems and Cephalosporins	Bloodstream infections.
Escherichia coli	Fluoroquinolones and Cephalosporins	Urinary tract infections.
Neisseria gonorrhoeae	Sulphonamides, Penicillin, Tetracyclines, Macrolides, Fluoroquinolones, and early generation Cephalosporins	Gonorrhoea

Table highlighting Multi Drug Resistant Bacteria

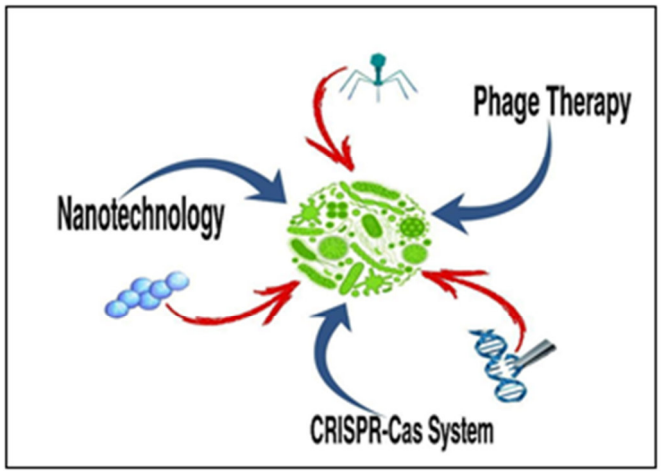

Fig 1: Technological tools to combat Multidrug Resistant Bacteria. More than one tool can be used.

Table 2. Mechanism of Resistance to common antibiotics[12-17]

Class of Antibiotics	Examples from the class of Antibiotics	Modes of Resistance
β lactam antibiotics	Penicillins, Cephalosporin and Monobactam	β lactamase, hydrolysis, degrading enzymes
Aminoglycosides	Neomycin, Paromycin, Ribostamycin, Amikacin, Gentamycin	Ribosomal mutation, Ribosomal modification by Methyltransferases, A-G modifying enzymes, cell membrane modification, Efflux pumps.
Tetracyclines	Limecycline, Clomocycline, Minocycline, Methacycline, Tigecycline	Ribosomal binding site mutation, Ara C transcriptional activators for development of resistance, Lon Protease, Intrinsic efflux of Tetracycline,
Macrolides	Josamycin, Midecamycin, Miocamycin, Rokitamyin, and Spiramycin	Ribosomal methylation, Antibiotic efflux, Target mutation, Drug modification,
Glycopeptides	Vancomycin, Teicoplanin	Van A and Van B and 9 resistant operons have been identified, morphological changes in cell wall synthesis, thickening of cell wall, reduce autolysis and change in content of Teichoic acid.
Quinolones	Norfloxacin, Ofloxacin	Alteration in target enzyme, Altered Drug Permeation, Alternate permeation of drug, Plasmid mediated Quinolone enzyme, Qnr plasmids, Acetylation by AAC(6')-Ib-cr
Sulfonamides	Sulfamethoxazole	Chromosomal Sul Resistance like mutation in dhps gene, plasmid borne DHFR (Dihydrofolate reductase) resistance, resistance by horizontal gene transfer, Cassette mediated resistance.

Table explaining the mechanism of resistance to common Antibiotics

1.1.1 Phage Therapy As A Promising Agent To Combat Multidrug-Resistant Bacteria

Bacteriophages are viruses composed of DNA or RNA and viral proteins and vary in their genetic diversity and complexity which infect bacteria using different mechanisms. They are pervasive, present in large concentrations in environmental sources like the sea, marsh, sewage [18]. Wherever bacteria are present in high quantities, they constitute the next layer of the human microbiota, infecting normal microflora of the human digestive tract and other niches. Bacteriophages can be lysogenic or lytic [19]. Lysogenic phages can integrated into the chromosome of the bacterial cell. Lytic phages infect the bacterial cell by attachment to particular receptors, replicate and assemble in the cellular cytoplasm, lyse the cells and release their offspring, which can further infect additional targeted bacteria. From the perspective of antibacterial agents, bacteriophages can be easily differentiated from different characteristic features including the production of Virolysin, antimicrobial peptide encoding, distributing system for genes encoding antimicrobial agents, causing infection to sensitive bacteria in the form of a living phage [20]. Various Phages and their mode of action against Multi-Drug Resistant Bacteria is given in Table 3.

1.1.2 Therapy By Using Virolysin

A significant type of bacterial cell wall hydrolases are Virolysins that helps to release phages through the degradation of peptidoglycan in the bacterial cell wall. Virolysins are encoded by the Lytic double-stranded phages

as the final stages of the phage lytic cycle [21]. Virolysins performs the bacterial cell wall hydrolysis mechanism in two steps: The first step comprises the proper binding to the specific sites on the bacterial cell wall followed by the second step which comprise *Streptococcus pneumoniae, Staphylococcus aureus, Bacillus anthracis.* Thus, this the has very high potential in overcoming Multi Drug Resistance as immunogenicity has least effect in its efficacy. Various studies reveal that the development of enzyme-resistant strains of pathogenic bacteria gets inhibited a promising therapeutic option for many pathogenic multidrug resistant bacteria such as: *Streptococcus* process delivers antimicrobial genes into intracellular bacterial pathogenic cells [38].

1.1.3 Therapy By Using Phage-Encoded Antimicrobial Peptides

Two types of antimicrobial peptides such as lytic factors and phage tailed complexes are encoded by bacteriophages. The former functions as an inducer that induces bacteriolysis at a particular time. Various lytic factors are there, such as E lytic factor and L lytic factor encoded by φX174 and MS2/GA classes of RNA phages respectively. The latter works through scanning of specific receptors on the bacterial cell surface, once they identify gets penetrate through the outer membrane, resulting in lysis of peptidoglycan followed by successful incorporation of the phage genome inside the bacterial cell. The tail of bacteriophage T4 [28-31] serves as the best example. Further, research is still needed in this area [32-34].

1.1.4 Phages Serving As A Therapy Delivery System

Significant viral delivery systems are developed nowadays for the insertion of the proper genome to the target cells [35]. One such efficient delivery system consists of phages that deliver genes encoding antimicrobials into target bacterial cells [36,37]. Hence, this

1.1.5 Combating Therapy Using Living Phages

All phages are not appropriate for phage therapy. Lytic phages are preferred as compared to the lysogenic phages since lytic phages are capable of rapidly infecting their hosts followed by fast replication which results in the production of a lot of progeny phage cells which lyse the bacterial cells. A survey was conducted to see the efficacy of phage therapy with a suppurative bacterial infection on 370 cases in which positive therapeutic results were obtained in 342 cases, confirming the efficacy of bacteriophages in the treatment of septic infection, caused by *Staphylococci*, *Escherichia*, *Klebsiella*, *Proteus*[38].

1.1.6 Alteration Of Biofilm Formation

Drug resistance in Bacteria is sometimes enhanced due to the presence of Biofilm formation. Thick biofilm allows lesser penetration of the antibiotics rendering the bacteria resistant to it. However, Bacteriophage can express certain enzymes like EPS depolymerase on the surface of the capsid that can degrade extracellular polymeric surface reducing the extent of surface colonization allowing the phage to access the bacteria associated with the EPS matrix. Complete removal of bacteria by biofilm inhibition is rare and usually bacteria are regrown after removal of antibiotic treatment. Evidence revealed phage treatment has resulted in complete removal of biofilms from various bacteria namely *Pseudomonas aeruginosa*, *Staphylococcus epidermidis* and *Listeria monocytogenes*[39].

1.1.7 Phage Cocktails

Phage cocktail preparation can be an alternative strategy to combat Multi Drug Resistant Bacteria however, due to the huge diversity of bacteriophage, designing a phage cocktail for optimal effect is more complicated than going for combinatorial treatment of antibiotics. Success of the phage therapy depends upon correct composition of Phage cocktails. The most important fact needs to be resolved is the selection of standardized cocktail or customized phage cocktail for the Phage Therapy. Customizing phage cocktails to each type of infection is time consuming whereas taking different spectrum into account standard phage cocktails with constancy in the type of phages may not bring out optimal clinical results due to non-specificity of phages to bacterial isolates. Novel approaches for Phage cocktail design consist of phages which act on the virulence factors reducing them making bacteria sensitive to the lytic phages present in the cocktail[39].

Table 3. Major phages under Experimental studies and their mode of action[40-41]

Phages	Types of Resistant Bacteria	Outcomes of Experimental Study
ØA392	*Pseudomonas aeruginosa* resistant to Imipenem	Mortality reduced in animals treated with bacterial specific virulent phage strain
LS2a	Drug resistant strain of *Staphylococcus aureus*	Abscess formation inhibited in Rabbit when phage was simultaneously inoculated *Staphylococcus aureus*
PS5	Multi Drug Resistant *Pseudomonas aeruginosa*	Deep wound infection was treated by topical application of the Phage
WP1, WP2, WP3, WP4, and WP5	XDR and MDR strain of *Pseudomonas aeruginosa*	WP2, WP3,WP4 conferred highest lytic activity against *Pseudomonas aeruginosa*
ϕMR11	MDR strain of *Staphylococcus aureus*	Able to efficiently eradicate MRSA from mice.
pVp-1	MDR strain of *Vibrio. parahaemolyticus*	Mice treated with the specific phage displayed protection from *Vibrio. parahaemolyticus* and survived intraperitoneal and oral challenges with the bacteria.
Biophage-PA	MDR *Pseudomonas aeruginosa* associated with chronic otitis	Count of *Pseudomonas aeruginosa* is relatively lower in the treated group with respect to the placebo group.
PEV20	*Pseudomonas aeruginosa* isolated from patient with cystic fibrosis and wound	Ciprofloxacin in conjunction with PEV20 inhibited the biofilm formation by the bacteria.

	infection.	
ZCKP1	*Klebsiella pneumonia* isolated from foot wound of a diabetic patient.	Increasing the multiplicity of infection results in decrease in bacterial count and formation of biofilm.
MSa	Methicillin Resistant *Staphylococcus aureus*.	Phage successfully killed the bacteria, prevented the formation of abscess resulting in reduction of bacterial load.

Table explaining the mode of action of various Bacteriophages against Multi Drug Resistant Bacteria

Table 4: Role of different Bacteriophages to combat Multi Drug Resistant Bacteria[42-45].

Sl. No.	Phage	Bacteria	Places where clinical trials are conducted
1	PBAB08 and PBAB25	*Acinetobacter baumannii*	South Korea
2.	BC-BP-01 to BC-BP-06,15 NCIMB deposit numbers 41174–41179	*Pseudomonas aeruginosa*	UCL Ear Institute and Royal National Throat, Nose and Ear Hospital, Nottingham, UK
3.	BPA43	*Klebsiella pneumoniae* subsp. *pneumoniae*	Hisar, Haryana
4.	WCHABP1 and WCHABP12	*Acinetobacter baumannii*	West China Hospital, Sichuan University, Chengdu, China
5.	KpJH46Φ2	*Klebsiella pneumoniae*	Mayo Clinic, Rochester, Minnesota, USA

Table showing various Phage whose clinical trials are conducted and clinically proved to be effective against Multi Drug Resistant Bacteria.

1.1.8 Advantages And Disadvantages Of Phage Therapy

Phage therapy has various advantages over traditional antibiotic therapy. Isolation of Phage is easier because of their ubiquitous distribution and they are absolutely abundant in every ecological niche which reduces their production cost with respect to the antibiotics. The locations where one can isolate bacteriophage are soil, water, hospital effluent, sewage effluent, hot spring, faecal material and also human and animals' gastrointestinal tracts. Moreover, Phage therapy may contribute to reduction of inflammatory response due to decrease in mean C-reactive protein and Leucocyte count making it one of the promising alternatives to Antibiotic treatment. Bacteriophages are highly specific to a particular bacterial strain hence they don't affect the normal microbial flora as compared to the antibiotics which may result in various superinfections and complications. Due to their innate self-replicating property the concentration of phages usually increases at the site of the infection preventing the growth of secondary pathogens which lowers the requirement for application of multiple doses to cure the disease. Other advantages of the phage are the absence of cross-resistance to the antibiotics. Though there are few disadvantages such as development of bacterial resistance to the phage, application of novel phage cocktails can be a potential solution to this problem. In absence of host phage doesn't multiply moreover phage may sometimes carry virulence factors and antibiotic resistant genes. Though there are clinically established bacteriophages against Multi Drug Resistant bacteria (table 4) but due to its various disadvantages phages are not accepted as pharmaceutical drugs hence efficient research needs to be conducted in this field to make this innovative tool functional for eradicating Multi Drug Resistant Bacteria from the society.

2. USE OF NANOTECHNOLOGY TO COMBAT MULTIDRUG-RESISTANT BACTERIA

2.1 *Silver Nanoparticles As Nano-Bactericidal*

Silver nanoparticles are one of the most studied and used metal nanoparticles as an effective antimicrobial agent [46]. The bactericidal mechanism of silver nanoparticles initiates through anchoring and penetration of Gram-negative bacteria's cell wall which leads to structural changes in the morphology of the cell membrane, resulting in increased membrane permeability that changes the transport pathways through plasma membrane leading to cell death [47].Thiol groups of vital enzymes and the phosphate groups of DNA interact more with silver nanoparticles leading to inhibition of DNA replication followed by cell death [48,49].The free radical formation is linked with the antimicrobial mechanism of silver nanoparticles which results in induced membrane damage [50]. This has been applied to Gram positive as well as Gram negative bacteria. It has been shown that physicochemical properties exhibited by nanoparticles edited the bactericidal effect [51]. Silver nanoparticles using biosynthetic machineries like fungus, yeast, bacteria and plant extracts possess strong antibacterial efficacy against many multidrug resistant pathogens like *Mycobacterium tuberculosis*, *Pseudomonas aeruginosa*, *Streptococcus pneumoniae*, Methicillin-resistant *Staphylococcus aureus*, *Klebsiella pneumoniae*. Biologically synthesized silver nanoparticles used against the standard strain of *Mycobacterium tuberculosis* and 26 clinical isolates including multidrug resistant (MDR) strains were evaluated [52]. Various clinically approved Nanoparticles was found to be effective against Multi Drug Resistant Bacteria (Table 5).

2.2 *Nanoprisms As Nano-Bactericidal*

Nanoprisms show efficacy in combating methicillin-resistant *Staphylococcus aureus* [51]. The nano prisms work through different mechanisms as they possess different crystalline planes with different surface energy having variations in surface reactivity, thereby releasing silver nanoparticles from the tips and edges to promote efficient bactericidal effect.

2.3 *Nitric Oxide Releasing Nanoparticles As Nano-Bactericidal*

Broad spectrum antibacterial activity is possessed by iron oxide nanoparticles. It can inhibit the growth of many antibiotic resistant bacteria such as *Klebsiella pneumoniae*, *Enterococcus faecalis*, *E. coli*. NO is a natural gas that is lipophilic and hydrophilic in nature, being unstable in an

oxygen environment [53]. The reaction between nitric oxide and oxygen or superoxide produces reactive oxygen as well as nitrogen intermediate products that are toxic to the cell, thus acting as an antimicrobial agent. The reactive nitrogen oxide species (RNOS) like peroxynitrite (OONO−) [54]. Lipid peroxidation of liposomes is mediated by Peroxynitrite, which contributes to the antimicrobial activities of nitric oxide nanoparticles [55]. Autoxidation of nitric oxide causes DNA damage through RNOS where deamination of cytosine, adenine, guanine occurs [56]. It also inhibits DNA repair enzymes that are associated with the repair of alkylation to DNA [57]. Further, it has been reported that this method is efficient against *methicillin-resistant Staphylococcus aureus (MRSA)* and *Acinetobacter baumannii*.

2.4 Zinc Oxide Nanoparticles As Antibacterial Agent

Zinc oxide nanoparticles exhibit good antibacterial properties [58] such as photocatalytic activity which is attributed to the generation of reactive oxygen species (ROS) [59,60]. Toxic effects have been shown by zinc oxide nanoparticles to (methicillin)-resistant bacterial strains such as *Staphylococcus aureus* and *Streptococcus agalactiae* [61]. The mechanism through which nanoparticles works initiates with its internalization inside the cell which increases the oxidative stress and causes damage to all the components of cell including proteins, lipid and DNA [61] resulting in disorganization of cell wall followed by damage of cell membrane. The toxicity of zinc nanoparticles depends upon concentration and is very little toxic at low concentrations. Zinc oxide nanoparticles exhibit antibacterial properties against Gram-positive as well as Gram-negative microorganisms [62]. These nanoparticles are also effective against extended-spectrum β lactamases-producing *E coli* and *Klebsiella pneumonia* [63].

2.5 Titanium Dioxide, A Nanocomposite As Antibacterial Agent

As a substitute for metal nanoparticles, metal oxide nanoparticles have also been widely used as an antimicrobial agent. Titanium dioxide is one of the most commonly used non-silver nanoparticles [64–67]. Its antibacterial action is photo dependent, consequently generates free radicals during photocatalytic reactions. These free radicals operate further through degradation of lipopolysaccharide, peptidoglycan, phospholipids bilayer owing to peroxidation in the bacterial cell. The efficacy of twenty-two different antibiotics with titanium dioxide nanoparticles has been studied [68]. Titanium dioxide particles target *S. mutants* and *A. actinomycetemcomitans*, both are multidrug resistant organism [66].

2.6 Copper Nanoparticles, A Nanocomposite As Antibacterial Agent

The mechanism of action of copper nanoparticles is based on the release of Cu (II) ions on contact with moisture from the nanoparticles itself. These copper ions then bind with the –SH and –COOH groups of protein molecules of the bacterial cell wall for further processing. Copper nanoparticles target *A. baumannii*, a multidrug resistant organism [69].

2.7 Biofilm Formation And Quorum Sensing Inhibition By Nanoparticles

Many studies have revealed that surface-functionalized NPs combined with b-cyclodextrin (b-CD) are being able to interfere with the signalling molecules preventing the molecules interact with their cognate receptors therefore repressing the process of Quorum sensing and obstructing bacterial communication. Biofilm inhibition by gold NPs (AuNPs) has been reported in many papers. Recently Gopalakrishnan with his colleagues (Vinoj et al., 2015)[70] established AuNPs coated AiiA (N-acylated homoserine lactonase proteins) purified from *Bacillus licheniformis* were found to inhibit EPs production and antibiofilm activity against *Proteus* sp. at concentration of 2-8 μM. A recent study revealed by Yu. et al. that AuNPs can strongly attenuate Biofilm formation associated with *Pseudomonas aeruginosa*. The mechanism of inhibition was due to interruption of interaction mediated by adhesins between bacteria and the substrate surface due to electrostatic interactions established between AuNPs and the cell wall surface of *Pseudomonas aeruginosa*. Thus, the use of NPs demonstrates an innovative approach to penetrate the infectious biofilm targeting the bacterial communication resulting in prevention of major health issues associated with Multidrug Resistant Bacteria[71].

2.8 Antibiotic Conjugated Nanoparticles

Recent studies have highlighted a newer approach to combat multidrug resistance where vancomycin has been conjugated with nanoparticles have shown to enhance antibacterial efficacy against Vancomycin resistant *Staphylococcus aureus*[71]. Gu et al. in 2003 [72] have conjugated gold nanoparticles (Au@Van) with vancomycin and used it against Vancomycin resistant enterococcus and an increased antibacterial efficacy was established. Other formulation (VBGNPs) was tested against *Escherichia coli* and drug resistant strains of *S.aureus*. Another interesting formulation was antimicrobial activity of C-AuNp-Amp (Gold nanoparticle capped with chitosan and coupled with ampicillin), the application of this formulation resulted in two-fold increase in antimicrobial efficacy with respect to free ampicillin alone. Other examples are amino substituted pyrimidine do not possess any antibacterial activity but when coupled with Gold nanoparticles shows antibacterial activity against Multi Drug Resistant isolates of *Escherichia coli* and *Pseudomonas aeruginosa.*[73]

2.9 Nanotheranostics

The term Theranostics explains the combination of Diagnosis and Therapy into one single platform which results in bio detection and real time monitoring of the required therapy. The following strategy can be done in nanoscale and hence termed as Nanotheranostics. Research is conducted and many nanoplatforms are prepared to target drug resistance bacteria. A selenium nanoplatform (Se@PEP-Ru) was developed with potential fluorescent properties which can not only help in imaging bacteria but also confer efficient antimicrobial properties. Zhao and co-workers have developed an innovative theranostics nanoprobe for near-infrared fluorescence imaging and photothermal therapy for MRSA infection. Kuo and his co-workers contributed to the development of nano theranostics system using Au nanorods conjugated with a photosensitizer hydrophilic in nature which can serve as dual-function agents in photodynamic inactivation and hyperthermia against MRSA[71].

2.10 Lipid Polymer Nanoparticles

As previously discussed about the inhibition of biofilm formation as a novel strategy to combat Multi Drug Resistance[73]. Another approach that supports the hypothesis is the synthesis of Lipid Polymer nanoparticle by conjugation of Rhamnolipid a biosurfactant secreted by *Pseudomonas aeruginosa* to polymeric nanoparticle use to overcome resistance of *Helicobacter pylori*. The mentioned novel particulate system comprises chitosan polymer as a core of the structure having clarithromycin encapsulated in it and the shell is composed of 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[amino(polyethylene glycol)-2000) DSPE-PEG2000-decoratedrhamnolipids. The eradication of the microorganism was enhanced as the composition of Rhamnolipid was enhanced resulting in considerable reduction of biofilm biomass and viability.

2.11 Anti-Microbial Oligonucleotides

Anti-microbial oligonucleotides are Transcriptional factor Decoys (TFD) which are found to be effective against Multi Drug Resistant bacteria. These are very short fragments of oligonucleotide specific to certain regions of the DNA capturing certain regulatory proteins to repress certain essential genes in the bacterial cell overcoming drug resistance[74]. One of the greatest challenges is the DNA encapsulation in a suitable carrier protecting it from nuclease degradation and targeting to the specific site of action. Gonzalez-Paredes et al. gave a solution by coating anionic solid lipid nanoparticles with protamine or cationic bola amphiphile 12-bis-tetrahydroacridinium. Both the compound shifted the zeta potential to positive values and revealed the protective effect of TFD from the attack of nuclease. Many authors reported other possibilities of conjugating oligonucleotide antimicrobials with various cationic materials like peptides for penetrating the cell.

2.12 Cationic Peptides

Inclusion of Cationic peptides can be an alternative option to conventional antibiotics. The unique features of these peptides are their amphiphilic nature and their cationic charge, which help them to target negatively charged bacterial membranes leading to release of intracellular contents and death[75]. As the restoration of cell structure is practically impossible hence the emergence of bacterial resistance can be successfully minimized.

2.13 Nano-Antibiotic

A new innovative method where the therapeutic agents are transformed into nano-sized assemblies, this may result in a carrier-free drug delivery approach. This approach can alter the physical properties of antibiotics, increasing their rate of dissolution, drug bioavailability, side effects potentially reduced, improved interaction and penetration within the bacterial membranes, thus can efficiently inhibit against antibiotic-resistant strains. Studies have revealed the effect of Clarithromycin nanocrystals towards *Helicobacter pylori*. The bioavailability and concentration of the drug at the desired site of action was better with respect to coarse clarithromycin powder. Hyperbranched polyester was developed to be a new nano-grade antibiotic thus overcoming the complication of antibiotic encapsulation[76].

Table 5 shows the role of Nanotechnology to combat MDR by using different nanoparticles [61,66,77,78].

Nanotechnology using nanoparticles	Particle size (nm)	Target MDR organisms	Place where clinical trials are conducted
Silver nanoparticles	5–100	Methicillin-resistant *Staphylococcus aureus*	University of Silesia, ul. Jagiellońska 4, 41-200 Sosnowiec, Poland
Nitric oxide releasing nanoparticles	20–100 (NO donor particle)	*Klebsiella pneumoniae, E. coli*	University of Michigan Medical School, Ann Arbor, Michigan
Zinc oxide	12–60	Methicillin resistant *Streptococcus agalactiae* and *Staphylococcus aureus*	University of Michigan; Ann Arbor, USA
Titanium oxide	20	*Staphylococcus aureus*	Plymouth University, Plymouth, Devon, PL6 8BU, UK
Copper nanoparticle–cotton composites	5 nm	*A.baumannii*	Uniformed Services University of the Health Sciences, Bethesda, Maryland, USA

Table explaining Nanotechnology and use of clinically approved nanoparticle to combat Multi Drug Resistant Bacteria.

2.14 Use Of Crispr-Cas System To Combat Multidrug-Resistant Bacteria

Genome editing has transformed the modern world by the availability of genome editing which edits the genomes of organisms meticulously [79]. The CRISPR-Cas system is one of the most widely used genome editing tools. The emergence of Clustered Regularly Interspersed Short Palindromic Repeat (CRISPR) that function with CRISPR Associated (Cas) proteins as (CRISPR)-Cas9 system is an RNA guided endonuclease that targets DNA to knock-on and knock-out DNA specific antibiotic target sites. [80] This system shows enormous applications in various fields like in the treatment of genetic diseases [81], to perform genome engineering of many bacteria [82], plants [83], mice [84], also antibiotic resistance is reversed in different multidrug resistant bacteria through successful targeting of resistance genes. [85] Sometimes, it functions as a molecular recording device [86]. This genome editing system is found in bacterial genomes as well as archaeal genomes around 50% and 87% respectively [87]. The genetic loci of the CRISPR-Cas systems comprise the CRISPR array, which consists of short repeated sequences (repeats) and similarly sized flanking sequences (spacers). CRISPR array spacers are known as protospacers, which are derived from DNA sequences from invasive phage or plasmid. Cas proteins are essential functional elements of CRISPR systems

that are encoded upstream of the CRISPR array for determining the behaviour of the system [88].The CRISPR-Cas system is used efficiently to combat multidrug-resistant *Staphylococcus aureus* (MRSA), a dangerous human pathogen that is resistant to β -lactam antibiotics [89]. The pathogen is resistant since the mecA methicillin resistance gene is present which codes for penicillin-binding protein 2A, resulting in the inhibition of the activity of β -lactam antibiotics. To combat this MRSA, the promoter region of mecA in MRSA is targeted by the electroporation technique to introduce the effector plasmid vectors and oligonucleotides for the suppression of transcription. This suppression mechanism requires the designing of CRISPR-dCas9 system [90]. The mechanism of CRISPR systems is similar to RNA interference (RNAi) in eukaryotic cells, that use small RNAs (sRNAs) to identify and neutralize. In short, the CRISPR- dCas9 system requires the creation of the RNA Guide (gRNA), including a target-specific nucleotide spacer (~20nt) and an endonuclease of Cas9 [91]. The gRNA signals Cas9 to the target DNA, to produce a double-stranded break [92]. The pathway of homologous repair or non-homologous end junction is used to resolve this split. The former works based on an error-prone mechanism that can knock out the gene by a combination of absurd-mediated decay of the mRNA transcript and pre-maturity truncation of protein mechanisms, a process that is not always especially successful while the latter works on another method for fixing a double-strand break in DNA by introducing a particular mutation with the insertion of a homologous piece of DNA [93]. Mutants are produced through these processes. Additionally, this device may be used for activation as well as inhibition of transcription by using the catalytically dead Cas9 (dCas9). One of the commonly utilized expression tools for MecA gene expression levels is Reverse Transcriptase Quantitative Real-Time Polymerase Chain Reaction (RT-qPCR) [93]. By analyzing with this tool, it was known that the expression of the mecA gene in the CRISPR-treated sample was reduced which reflects a positive decrease in gene transcription, thus combating multidrug- resistant bacteria. The CRISPR-Cas system provides new insights for the elimination of MDR pathogens, making differentiation between beneficial and pathogenic microorganisms (Table 6).

Table 6 Role of CRISPR-Cas system to combat MDR [94-99].

Sl. No.	Phage/ plasmid	Bacteria	Places where clinical training are conducted
1.	*Staphylococcus aureus* strain RF122 ϕSaBov-Cas9-nuc	*Staphylococcus aureus* str ain ATCC 6538	Mississippi State University, Mississippi State, Mississippi, United States of America
2.	plasmid pRESAFRESBL	*Escherichia coli K12 BW2513*	Sungkyunkwan University, Suwon 16419, Republic of Korea
3.	Plasmid pKH88 [sp-ermB]	*Enterococcus faecalis*	Department of Biological Sciences, The University of Texas at Dallas, Richardson, Texas, USA Department of Immunology & Microbiology, The University of Colorado School of Medicine, Aurora, Colorado, USA
4.	Plasmid pSH12	*Clostridium difficile*	Auburn University, Auburn, AL, USA Guizhou Medical University, Ministry of Education, Guiyang, People's Republic of China
5.	plasmids pVPL3004 and pVDM10001	*Enterococcus faecium* strain E745	Department of Medical Microbiology, University Medical Centre Utrecht, Utrecht, The Netherlands. Department of Food Science, University of Wisconsin-Madison, Madison WI, United States of America. College of Medical and Dental Sciences Institute of Microbiology and Infection, University of Birmingham, Birmingham, United Kingdom.

Table shows the application of CRISPR-Cas system under clinical trials used for combating Multi Drug Resistant Bacteria.

2.15 Antimicrobial Peptides

Recently many studies are being conducted to overcome the problem of Multi Drug Resistance and it was treated as a potential alternative to conventional antibiotics[100]. Antimicrobial peptide confers weak antimicrobial activity but strong immunomodulatory activity when the host organism is invaded by pathogenic microorganisms. They are sometimes termed as "host-defence" peptides, they are unable to activate the adaptive immune system but modulate the immune system through adjuvant-like activity. Report of bacteria getting resistant to antimicrobial peptides by altering the charge of surface molecules or proteolytic cleavage by secreting extracellular protease is rare and takes a long period when compared with conventional antibiotics. Antimicrobial peptides incur more cost with respect to antibiotics but studies revealed that antimicrobial peptides can act in a synergistic fashion when applied with antibiotics[100].

3. ANTIMICROBIAL PEPTIDES AND ITS MODE OF ACTION

3.1 Effect On Cell Wall Lipid II

Production of bacterial cell wall also termed as peptidoglycan can be inhibited resulting in development of resistance in bacteria against β lactam antibiotic such as penicillin. MRSA was found to have penicillin-binding protein 2a (PBP 2a) which was absent in susceptible *Staphylococcus aureus*.

Vancomycin resistance was developed due to the presence of depsipeptide D-Ala-D-Ala in the peptidoglycan. However antimicrobial peptides having unusual amino acids also termed as Lantibiotics may exert antibacterial activity via interaction with the cell wall components. Lantibiotics are antimicrobial peptides which are ribosomal-synthesized and post translationally modified peptides that consist of intramolecular ring structure usually produced by Gram positive bacteria and acts on broad range of bacteria comprising both Gram positive and Gram negative in nature. Lantibiotics are classified into either type-A or type-B that can damage bacterial membranes and inhibit production of enzymes respectively. Examples of Type A-Lantibiotics include Subtilin, epidermin, nisin and Pep5 whereas Type-B include Cinnamycin and Metsacidin. Recently studies revealed nisin can produce transient pores that results in cytoplasmic membranes to be permeable. Subtilin permeabilizes lipid containing membrane in lipid II dependent manner [101,102].

3.2 Ameliorating The Membrane Potential For Induction Of Membrane Permeabilization

Major two mechanisms of multidrug resistance are phenotypic changes in microbes under certain growth conditions and lesser accumulation of antibiotics due to nonspecific pumping out of drugs by the efflux transporter proteins. The most common mechanism of evading the action of antibiotics is due to disruption of cytoplasmic membrane by formation of pore through mechanisms of barrel-stave, toroidal pore or through a non-pore carpet like mechanism. Furthermore, antimicrobial peptides should be able to permeate the cell wall and the plasma membrane to reach their desired intracellular targets such as nucleic acids and functional proteins. In the barrel-stave model channel forming peptides of variable number are positioned in a barrel-like ring surrounding an aqueous pore. These types of transmembrane pores are induced by Alamethicin and Ceratotxin[103]. In the Carpet model, antimicrobial peptides start accumulating on the membrane surface forming an electrostatic interaction with the anionic phospholipid heads of the plasma membrane carpeting various sites of the membrane. At the verge of reaching the threshold concentration of the phospholipid, disruption of membrane occurs in a detergent like manner without any formation of pores. Cercopin P1 and Caerin 1.1 are the examples of antimicrobial peptides following the carpet model of membrane disruption[104]. Toroidal pore model involves the antimicrobial peptide associated with the phospholipid head group regions of the bilayer resulting in the induction of high curvature fold in the bilayer resulting in both the leaflets of the bilayer communicate directly at a torus which is lined by the leaflets. Examples are Magainin, Cathelicidin and HPA3[102].

4. FUTURE DIRECTIONS

Phages possess differences in biological, physical, and pharmacological properties aa compared to conventional antimicrobials, and need attention. Phages are highly specific so there is a need to employ multiple phages isolates for more efficient treatment. Alternative approval pathways required to be addressed for phage therapy [105]. Clues have been provided from the in vivo and in vitro studies related to the specific mechanisms on which nanotechnology works on, as nanoparticles trigger an adverse effect which enlightened the future surface modification of nanoparticles to make them less toxic and safer [106]. These concerns are related to nano safety and need to be addressed. In vitro methods to establish the toxicological profile of nanoparticles are needed for the classification according to the data derived from this profiling. These efforts might provide information on the concentration of nanoparticles that should be taken for safe medical purposes. The CRISPR-Cas system can prompt off-target effects, likely resulting in damaging outcomes. To overcome this, many techniques are still left to be devised in the near future. A proper tool for the selection of sgRNAs should be designed. Also, an effective delivery system with fewer somaclonal variations is required to be developed. Furthermore, more research is required to be conducted to increase on-target efficacy with minimal off-target effects [106]. The current momentum to soothsay the 'future of CRISPR' lies in controlling the composition of the microbial community to being utilized as a conventional broad-spectrum antibiotic.

5. CONCLUSION

The strategies addressed in this present review can include new ways to combat the multidrug resistant bacteria. In summery one can conclude that one possible line of study to fight against the deadly Multi Drug Resistant bacteria is in analysing several strategies associated with molecular mechanism of Multi Drug Resistance. In this context many clinically approved bacteriophages were found to be effective against various strains of MDR and this method had gained importance due to lack of immunogenicity in human host. Even scientific findings on evolution of nanoparticles and nano formulations against Multi Drug Resistant bacteria led to a new innovative method where nano-sized assemblies resulted in a carrier free drug delivery approach. Further highlights on CRISPR-Cas system and Antimicrobial peptides led to a positive insight for elimination of MDR pathogen from environment. However, the techniques require further research for their optimization and fruitful application.

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7. AUTHORS CONTRIBUTION STATEMENT

Ms Tamalika Chakraborty and Dr. Sumana Chatterjee conceptualized and gathered the data with regards to this work. Ms Ranjana Shaw put necessary inputs towards designing of the manuscript. All authors including Dr. Lopamudra Datta and Dr. Abhijit Sengupta discussed the results and conclusion and contributed to the final manuscript.

8. CONFLICT OF INTEREST

Conflict of interest declared none.

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