Acinetobacter Baumannii Infections

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Antibiotic resistance is one of the biggest challenges for the clinical sector and industry, environment and societal development. One of the most important pathogens responsible for severe nosocomial infections is *Acinetobacter baumannii*, a Gram-negative bacterium from the Moraxellaceae family, due to its various resistance mechanisms. The enormous adaptive capacity of *A. baumannii* and the acquisition and transfer of antibiotic resistance determinants contribute to the ineffectiveness of most current therapeutic strategies, including last-line or combined antibiotic therapy. In this review, we will present the current progress in developing innovative strategies for combating multidrug-resistant *A. baumannii* (MDRAB) infections.

Keywords: antimicrobial resistance ; β-lactamases ; Acinetobacter baumannii ; antimicrobial peptide ; bacteriophage ; CRISPR

1. Introduction

In recent years, the resistance phenomenon was encountered in most common bacterial strains causing infections, associated with an increased risk of morbidity, mortality, high treatment costs and long periods of hospitalization. One of the ESKCAPE pathogens responsible for nosocomial and community-acquired infections is *Acinetobacter baumannii*, a Gram-negative, non-motile, non-fermentative and non-sporulated bacterium from the *Moraxellaceae* family [1]. Two of the many reasons for the success of MDRAB strains are the association with chronic nosocomial infections and their unique ability to survive in extreme environmental conditions. Its reputation is mainly due to its association with severe infections caused to the US military during the wars in Afghanistan and Iraq, which is why it has been called “Iraqibacter” [2]. *A. baumannii* causes various infections, including pneumonia, urinary tract infections, skin and soft tissue infections or nosocomial meningitis [3]. Due to its extended resistome and virolome, evasion of the host's immune effectors, ability to grow in biofilms, to survive in extreme environmental conditions, and to switch to latent growth forms with a minimal metabolic rate, the treatment options are limited, rendering *A. baumannii* one of the most critical and fearful pathogens [4] [5].

In this review, we will present an update regarding the perspectives of new therapeutic strategies efficient against MDRAB. In addition, the discussion section will present the main challenges of therapeutic strategies and the need for further studies in response to existing limitations.

2. Innovative Strategies for Treatment of *A. baumannii* Infections

Studies conducted in recent years highlight the unique involvement of *A. baumannii* strains in increasing the severity of nosocomial infections and, implicitly, their associated morbidity and mortality rates. Given that *A. baumannii* strains are resistant to almost all antibiotics used [6], the research direction must be in line with the “post-antibiotic era”, emphasizing the development of innovative strategies to control MDRAB spreading. Next, we will present the most innovative therapies, such as phage therapy, new antimicrobial peptides, and CRISPR Cas system (Clustered regularly interspaced short palindromic repeats) developed to prevent the spread of MDRAB strains.

2.1. Bacteriophages Therapy

Bacteriophages are viral parasites able to infect bacteria by recognizing surface receptors, injecting their genetic material into the host and replicating using the host cellular machinery [7]. Phages exhibit ecological and genetic effects on bacteria at the population level, and these effects can impact plasmid stability [8][9]. Phages may enhance the persistence of ARGs as an adaptation strategy to restrictive environmental conditions, e.g., wastewater aggressively treated using UV, temperature or pH. However, genetically modified phages could be used to increase antibiotic susceptibility of resistant strains. The alarming increase in the resistance rates has also led to the revival of phage therapy to increase the

susceptibility level of bacteria by eliminating resistance and virulence markers [10]. In addition, research has shown that phage therapy has a high potential to represent an effective and safe treatment against MDRAB strains [12][13]. A high number of experiments were performed both in vitro and in vivo, the main results being summarized in Table 1.

Table 1. Bacteriophages therapy against *A. baumannii* strains.

Phages	Family	Isolation Source	Type of Study	Number of Tested Strains	% of Susceptible Strains	Animal Model Application	References
økm18p	Corticoviridae	hospital sewage	in vitro	34 MDR, 16 of those XDRAB	44.1%	NA	[14]
Acibel004	Myoviridae	wastewater sample	in vitro	34 MDR	82.3%	NA	[15]
Acibel007	Podoviridae	wastewater sample	in vitro	34 MDR	82.3%	NA	
IsfAB78	Myoviridae	water sample	in vitro	43 MDR	27.9%	NA	[16]
IsfAB39	Podoviridae	water sample	in vitro	43 MDR	25.5%	NA	
vB_AbaS_Loki	Siphoviridae	sludge	in vitro	34	5.8%	NA	[17]
Petty phage	Podoviridae	sewage	in vitro	40, 25 of those MDR	10%	NA	[18]
SH-Ab 15599	Myoviridae	sewage	in vitro	48 CRAB	27%	NA	
SH-Ab15708	Myoviridae	sewage	in vitro	48 CRAB	29.1%	NA	
SH-Ab15497	Siphoviridae	sewage	in vitro	48 CRAB	29.1%	NA	[19]
SH-Ab15519	Podoviridae	sewage	in vivo	48 CRAB	16.6%	Mouse model—lung infection; 90% survival rate	

Phages	Family	Isolation Source	Type of Study	Number of Tested Strains	% of Susceptible Strains	Animal Model Application	References
vBGEC_AbM-G7was (phiG7)	Myoviridae	sewage	in vivo	200	68%	Rats wound model; 100% survival rate	[20]
Abp1	Moraxelaceae	sewage	in vitro	20	NA	Hella cells infection protection assay; 100% protection and survival rate of Hella cells.	[21]
			in vivo	20		Mouse local and systemic infection model; 100% survival rate.	
PB AB08	Myoviridae	Bacteriophage Bank of Korea	in vivo	14 MDR	35.7%	Mice model—intranasal phage cocktail; 35% survival rate	[22]
PBAB25	Myoviridae	Bacteriophage Bank of Korea	in vivo	14 MDR	7.1%	Mice model—ntranasal phage cocktail; 35% survival rate.	
WCHABP1	Myoviridae	hospital sewage	in vivo	2 CRAB	NA	Galleria mellonela infection model; 75% survival rate after phage administration	[23]
WCHABP12	Myoviridae	hospital sewage	in vivo		NA		

Phages	Family	Isolation Source	Type of Study	Number of Tested Strains	% of Susceptible Strains	Animal Model Application	References
PD-6A3	Podoviridae	sewage	in vivo	552 MDR	32.4%	Sepsis mouse model; intraperitoneal administration; endolysin therapy, endolysin + phage therapy, phage therapy and phage cocktail; 70%, 70%, 60% and 50% survival rate.	[24]
			in vivo		NA	Galleria mellonella infection model; 80% and 50% survival rate at 96 and 48 h.	
Bϕ-R2096	Myoviridae	hospital sewage		20 CRAB		Mouse model acute pneumonia; 100%, 60% and 30% survival rate at day 12, with MOI 10, 1 and 0.1	[25]
			in vivo		NA		
AB3P1	NA	sewage, farm soil, feces of sheep, chicken litter, swab for surgical lounge.	in vivo	23	78.2%	Mice model; intraperitoneal administration of AB3 phages; 100% survival rate;	[26]

NA, not applicable; MOI = multiplicity of infection.

Bacteriophage therapy represents a promising tool in fighting MDR *A. baumannii* strains. Analyzation of the data summarized in Table 1 highlights that both *in vitro* and *in vivo* studies demonstrate the high efficiency, increasing the survival rate of organisms infected with *A. baumannii* strains. Based on the results obtained on animal models in the last ten years, numerous studies have focused on understanding the effectiveness of this therapy against chronic infections in the hospital units, as revealed by different clinical trials [10][27][28][29][30]. Schooley et al. have used phagotherapy in a patient with necrotic pancreatitis caused by an MDRAB strain [31].

Contrary to these studies, there are other reports of the inefficiency of phages in treating bacterial infections [32][33], which suggests that the clinical use of phages requires standardization. One of the most significant challenges in phage therapy is the resistance of bacterial strains to phage action [34][35][36]. In bacterial communities, resistance is a dynamic process when the antibacterial agent is biologic, as is the case with phages. On the other hand, phages exert a selective competition on bacteria. This two-way interaction causes a co-evolution that results in bacteria acquiring resistance mechanisms that can block the cycle of lytic infection [34][35]. Bacteria can acquire resistance to phages following cellular surface changes represented by point mutations in phage binding receptors [37]. Another mechanism of resistance is the outer membrane vesicles to which phages can bind due to surface structures, similar to those of parental cells. Binding of phages to these vesicles during invasion decreases the likelihood of cell infection [34]. In addition, a significant impact has the restriction–modification systems, the most common defense mechanisms in bacteria that can degrade foreign DNA, including double-stranded DNA phages [38]. Another concern in the use of phage therapy is the lack of standardization of phage preparation methods. An incomplete purification of host bacterial phages can lead to an unwanted transfer of bacterial toxins such as endotoxins or exotoxins [39]. Particular attention should be paid to combination therapy with phages and lysins. Once the dose needed to increase antimicrobial action has been determined, the mechanisms of action and elimination from the body must be established [40]. Stimulation by the phage of the immune response and adaptive immune systems, as well as their presence in the bloodstream, may influence the effectiveness of phage therapy [39].

Further studies are required to understand phage biology clearly and to better control clinical trials to standardize phagotherapy.

2.2. Antimicrobial Peptides (AMP)

Antimicrobial peptides may represent an alternative to antibiotics in the control of MDRAB strains spread. AMP is a class of compounds widespread in the living world as part of the innate immunity, acting as a primary barrier against infectious agents such as viruses, bacteria and fungi [41][42]. AMPs also play an essential role in regulating immune processes such as activating and recruiting immune system cells, angiogenesis and inflammation [43]. AMPs are amphipathic molecules with a positive electric charge, having a length of about 11–50 amino acid residues [43][44]. The main mechanisms of antimicrobial action of AMPs are the ability to cause cell membrane and cell wall damage, the inhibition of protein synthesis, nucleic acids and the induction of apoptosis and necrosis [45]. Due to these properties, AMPs have been considered an alternative to the use of antibiotics for limiting the spread and decreasing the infection rate and mortality control measures of nosocomial infections.

To be considered for therapy, AMPs must have a broad spectrum of action, high specificity and low cytotoxicity levels to mammalian cells [46]. The primary limitations that hinder the approval of systemic use of AMPs are sensitivity to enzymatic digestion and high toxicity, which is why most AMPs are applied topically and not orally or intravenously [47][48]. It has also been observed that certain physiological conditions, such as high concentrations of salts and serum components, can exert adverse effects on AMPs [49]. Compared to the conventional use of antibiotics, production costs for AMPs are much higher, which is why research is moving towards peptides as short as possible with stable properties [50]. Currently, research is aimed at developing technologies to improve the efficiency of AMPs in vivo, especially in terms of increasing the specificity against the infectious agents, decreasing cytotoxicity to mammalian cells, increasing stability and lowering production costs. The newest AMPs studied to elucidate their therapeutic efficacy against *A. baumannii* strains are summarised in **table 2.**

Table 2. Antimicrobial Peptides (AMPs) with antimicrobial activity against *A. baumannii*.

Organism	AMP	Type of Study	Animal Model	Main Results	References
NA	ZY4 cathelicidin-BF-15 derived	*in vitro; in vivo*	mouse septicemia infection model	Antibacterial activity in plasma; biofilm inhibition; kills persister cells; inhibition of infection and inflammation in vivo	[51]

Organism	AMP	Type of Study	Animal Model	Main Results	References
NA	epsilon-poly L-lysine (EPL)-catechol	*in vitro; in vivo*	mouse burn wounds infection model	Reducing bacterial burden in vivo	[52]
Vespa affinis	mastoparan-AF	*in vitro*	NA	Potent antimicrobial activity	[53]
NA	chex1-Arg20 amide (ARV-1502)	*in vivo*	Mouse infection model	Reduction of bacterial load	[54]
NA	α-helical -26 AMP residues	*in vitro*	NA	Great antimicrobial activity	[55]
Delftia spp.	delfibactin A	*in vitro*	NA	Great inhibitory effects	[56]
Capra hircus	mini-ChBac7.5Nα mini-ChBac7.5Nβ	*in vitro*	NA	Significant antimicrobial activity; induce membrane damages;	[57]
Hybrid striped bass Morone saxatilis × M. chrysops	I16 K-piscidine-1 analog	*in vitro; in vivo*	Sepsis mouse model	Strong bactericidal activity; high survival rate of infected mice;	[58]
Musca domestica	cecropin-4	*in vitro*	NA	Great bactericidal activity against MRAB and PRAB; inhibits biofilm formation	[59]
NA	Ω17 and Ω76 family peptides	*in vitro; in vivo*	Mouse peritoneal infection model	Disrupt bacterial membranes; induce small-molecule leakage; rapid bactericidal activity;	[60]
NA	ceragenins (AMP synthetic mimics)	*in vitro*	NA	Antibiofilm activity; inhibitory effects	[61]
Medicago truncatula	nodule-specific cysteine-rich (NCR) peptide and its derivatives	*in vitro*	NA	Potent killer of pathogenic bacteria	[62]
NA	TAT-RasGAP$_{317–326}$ anticancer peptide	*in vitro; in vivo*	Mousel model of lethal peritonitis	Growth inhibition effects; broad-spectrum antimicrobial activity; great efficacy in vivo	[63]

Organism	AMP	Type of Study	Animal Model	Main Results	References
NA	WLBU2-cationic amphipathic peptide	*in vitro*	NA	Eradicating bacterial biofilms;	[64]
Myxine glutinosa L.	myxinidin 2; myxinidin 3	*in vitro, in vivo*	Mouse skin wounds infection model	Antibiofilm activity; anti-inflammatory activity; enhance wound healing;	[65]
Hepatitis B virus	D-150–177C, HBcARD derivative peptide	*in vivo*	Mouse sepsis infection model	Strong bactericidal activity; 90% of mice protected from death;	[66]
Pisum sativum	nuripep 1653	*in vitro*	NA	Significant antimicrobial activity;	[67]
Cimex lectularius (bedbug)	CL defensin	*in vitro*	NA	Inducing membrane depolarization and pore forming; bactericidal action	[68]
Bungarus fasciatus	cathelicidin—BF derivate (Cath-A)	*in vitro*	NA	Bacterial growth inhibition	[69]
Lucilia sericata	LS-sarcotoxin and LS-stomoxyn	*in vitro; in vivo*	Mouse model infection	Strong activity against GRAM-NEGATIVE;	[70]
Leiurus quinquestriatus	venom cocktail proteins	*in vitro*	NA	Broad-spectrum antimicrobial activity; growth inhibition;	[71]
Myrmecia pilosula	Δ-Myrtoxin-Mp1a (Mp1a) heterodimeric peptide	*in vitro; in vivo*	Mouse model	Antibacterial activity; significant potency; nociceptive pain upon injection into mice	[72]
NA	glatiramer acetate	*in vitro*	NA	Efficient killing of clinical isolates	[73]
King cobra	OH-CATH30 D-OH-CATH30	*in vitro; in vivo*	Mouse model	Strong inhibition activity; low toxicity, great immunogenicity;	[74]
NA	stapled AMP Mag(i+4)1,15(A9 K, B21A, N22 K, S23 K)	*in vitro; in vivo*	Mouse peritonitis sepsis model	Great bactericidal activity; 88% of mice cured after intraperitoneal injection;	[75]
Viola odorata	Cy02 (cyclotide)	*in vitro*	NA	Strong bactericidal action	[76]

Organism	AMP	Type of Study	Animal Model	Main Results	References
P. aeruginosa bacteriophage	artilysin 175	*in vitro*	NA	High, rapid and broad antibacterial activity against MRAB	[77]
Calliphora vicina	FLIP 7	*in vitro*	NA	Antibiofilm activity	[78]
Camel (colostrum milk)	lactoperoxidase lactoferrin	*in vitro; in vivo*	Acute pneumonia mouse model	Major inhibition effects; significant clearance of A. baumannii in lung and blood culture;	[79]
Rana catesbeiana	ranalexin danalexin	*in vitro*	NA	Strong antimicrobial activity	[80]
NA	PNA $(RXR)_4$ XB	*in vitro; in vivo*	Galleria mellonella sepsis model	Excellent bactericidal activity in vitro; high dose of PNA conjugate required in sepsis model	[81]
NA	protegrin-1	*in vitro*	NA	Good activity against MRAB; no antibiofilm activity;	[82]
NA	aurein 1.2, CAMEL, citropin 1.1., LL-37, omiganan, r-omiganan, pexiganan and temporin A	*in vitro*	NA	CAMEL and pexiganan displayed the highest antibacterial activity	[83]

NA, not applicable; PRAB, polymyxin-resistant *A. baumannii*; HBcARD, human hepatitis B virus core protein arginine-rich domain; FLIP 7, fly larvae immune peptides 7; PNA $(RXR)_4$ XB, peptide nucleic acid conjugated with cell-penetrating peptide.

2.3. CRISPR System-a New Approach in the “Post-Antibiotic Era”?

As mentioned previously, the current strategies used to combat MDRAB infections have many limitations. In the recent years, one of the most attractive alternatives to combat bacterial resistance is the use of CRISPR (clustered regularly interspaced short palindromic repeat) system described for the first time in 1987, by Ishino et al [84]. CRISPR/Cas is an immune defense system encountered in bacteria able to recognize and degrade foreign nucleic acids through associated caspases.

One of the most significant advantages of this system is its high specificity, based on the existence of short, repetitive sequences in CRISPR loci separated from each other by single sequences of 26–72 pairs of lengths derived from MGEs such as plasmids or transposons [85]. The defense mechanism against exogenous genetic elements is accomplished in three stages: acquisition, expression and interference [86]. The acquisition stage involves the insertion into repetitive loci of the host chromosome of single sequences (spacers) derived from MGEs, separated by repetitive sequences. The expression consists of transcribing the complex formed of repetitive and spacer sequences into a single RNA transcript that will be further processed by caspases in short CRISPR RNAs. Caspases, including ribonucleases, are a family of protease enzymes, playing essential roles in programmed cell death [87]. In the interference phase, foreign nucleic acids are identified based on complementarity with CRISPR RNAs, and their degradation is accomplished by caspases [88]. Discrimination between self and non-self is accomplished through sequences from the foreign nucleic acid called

protospacers. These sequences are placed between some sequence motifs called PAMs (adjacent protospacer motif). Direct target recognition is achieved only by identifying these sequence motifs not stored in CRISPR loci, so there is no risk of degradation of its nucleic acid [89] (Figure 1).

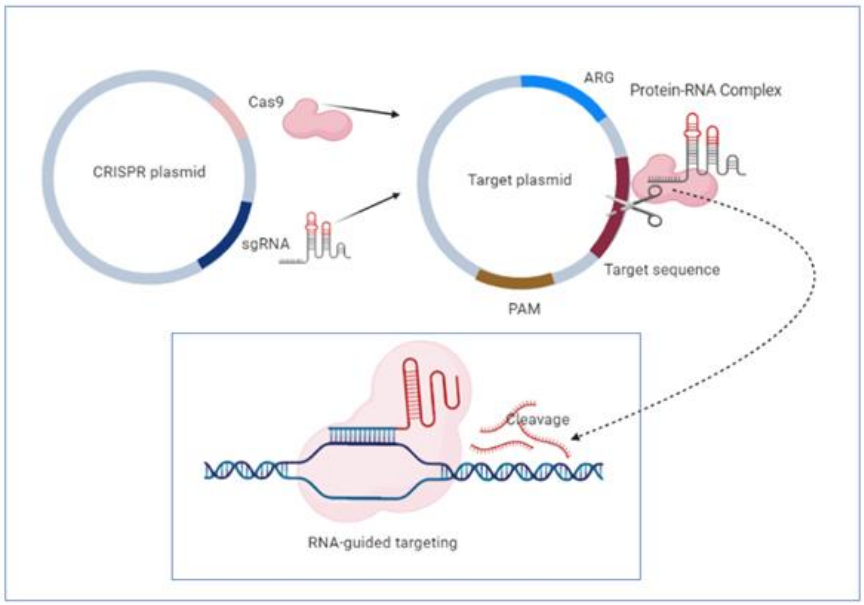

Figure 1. Schematic representation of clustered regularly interspaced short palindromic repeat (CRISPR)-based targeting of mobile genetic elements (MGEs). This system contains the cas9 nuclease, sgRNA transcript and other structural elements. In the first stage, sgRNA forms a complex with Cas 9 nuclease. The sgRNA transcript guide cas9 nuclease to introduce double-stranded breaks at the ends of the target DNA, leading to cleavage. Direct target recognition is achieved by recognizing protospacer adjacent motifs (PAM), short DNA sequences that are not found in CRISPR loci, so there is no risk of self-degradation. This system can be used to edit the genome of several antibiotic-resistant bacterial strains, leading to the removal of resistance determinants. Figure created with https://biorender.com/.

In *A. baumannii*, two types of CRISPR systems were identified within MGEs containing different spacer sequences. In addition, the distribution of some analyzed isolates in different clusters was observed, suggesting that this system was acquired by HGT throughout evolution [90][91]. Karah et al. analyzed 76 *A. baumannii* isolates to study the I-Fb subtype of the CRISPR system. Forty types of CRISPR sequences were revealed, two being found mostly in 35.52% of the analyzed isolates, suggesting the existence of two primary clones. The spacer sequences are arranged in chronological order of their inclusion from the invading nucleic acids so that the old ones are positioned at the end. This temporal positioning of the sequences allows the use of the CRISPR system for the micro- and macroepidemiological classification of clinical isolates [92].

These results have opened new perspectives regarding the possibility of using the CRISPR system for subtyping *A. baumannii* strains. Wang et al. developed a CRISPR platform that allows rapid genomic editing by introducing deletions, insertions, and point mutations to analyze the mechanisms involved in oxidative stress (OxyR) in *A. baumannii* strains. For the introduction of deletions, the authors constructed a CRISPR plasmid in which they incorporated the CRISPR elements from *Streptococcus pyogenes*. To repair double-strand breaks, they used RecA recombinase from *A. baumannii*. The introduced mutations in the oxyR gene and its deletion, lead to a high susceptibility level of *A. baumannii* strains to oxidative stress, demonstrating the importance of this gene as a central transcriptional regulator of the response to oxidative stress [93]. Mangas et al., conducted an in silico study to analyze the pan-genome of 2500 *A. baumannii* strains. Depending on the number of shared genes, the authors observed that genomes are divided into two broad groups. The group of strains with a lower number of genes shows the sequences and genes characteristic of the CRISPR system, genes specific to the toxin–antitoxin system and genes involved in the biofilm formation, which is why it is considered that the CRISPR system may have an essential role in virulence. Unlike the strains from group two, positive for a high number of plasmids, the strains from the first group contain mainly genes involved in regulating the elements of the CRISPR system. This finding led to the idea that the CRISPR system may be involved in the restriction of the plasmid entry into bacterial cells [94]. Karlapudi et al. conducted an in silico study to understand the role of the AbaI gene in biofilm formation in *A. baumannii*. For this, they used a series of genetic editing tools to create AbaI gene knockouts. The analyzed tools (CHOCHOP, CCTop, E-CRISP, CRISPR Direct, Off-Spotter, Crispr-era) can provide information about the target sequences, specific primers, existing mutations, the location of the target sequence in order to perform the knockout, as well as the necessary sgRNA sequences for performing genomic editing experiments [95]. The information obtained from the in silico genomic experiments demonstrates the need to improve genetic editing tools. In addition, further studies should consider the construction of sgRNA with a custom design, depending on the diversity of cell types.

Despite the notable results obtained from using the CRISPR system to combat antibiotic resistance, however, controlling bacterial populations using this strategy has some limitations. First of all, the appearance of mutations outside the target represents a significant limitation of the CRISPR system. In addition to cleaving target sequences, the CRISPR system can act on identical or homologous DNA sequences, leading to mutations in unwanted sites, called off-target mutations. Mutations outside the target can lead to cell death or transformation, which is why it is recommended to select target sites at which as few mutations can occur outside the target [96].

Another challenge in using the CRISPR system in controlling bacterial populations is the need for PAM sequences that are involved in differentiating between self and non-self. There is a limitation of the number of target sites due to the need for these sequences. Because the CRISPR system requires specific PAM sequences to function, their genetic engineering processing can eliminate this limitation [96].

Another major limitation in using the CRISPR system in the control of bacterial populations is represented by the delivery of the protein–RNA complex through the bacterial membrane. There is the problem of delivering the CRISPR system in the case of both Gram-negative and Gram-positive bacteria. It has been observed that the techniques used to encapsulate the gRNA-protein complex have a significant impact on loading and packaging efficiency, thus limiting their practical use [97][98]. Consequently, studies have focused on the use of phages as a vehicle for the delivery of the CRISPR system at the target level. Starting from phages as a delivery vehicle of the CRISPR system in vitro, the problem of controlling bacterial populations using the CRISPR system in vivo was raised. Starting from the fact that oral administration of phages for targeting bacteria in the intestinal tract was used successfully [99], one strategy is to use phages as a vehicle for delivering the CRISPR system in the intestinal microbiota, for eliminating the ARGs. However, it is required to have a collection of phages specially designed to target ARGs, to establish the optimal concentration required and to know the several barriers that occur in vivo, such as inactivation of bacteriophages by gastric acid, neutralization of phages by the spleen and the immune system [100].

3. Discussion

To date, a significant challenge in the clinic is to develop methods to establish the appropriate MIC values needed to increase treatment efficiency. Most laboratories are not able to determine MIC values accurately and reproducibly enough and to eliminate variations. One cause of variations in MIC values is the existence of several strategies and methods to determine these values. Terwee et al. studied the differences obtained in MIC values following the use of several methods that fall into two general categories: "anchor" methods and distribution-based methods. Anchor methods use an external criterion to establish a significant change (patient opinion), and distribution-based methods use statistical data to determine the MIC value. In the case of applying the two strategies, Terwee et al. observed significant differences in MIC values [101]. These significant differences could be closely related to population characteristics such as age, the severity of the condition and treatment and the method used. In this situation, several factors that contribute to variability make it difficult for clinicians to establish a single MIC value or at least a range of values as small as possible [101].The semiautomatic susceptibility detectors often provide truncated MIC values. The same problem exists with gradient tests, such as the E-test, which may omit a certain percentage of resistant strains, leading to treatment failure in the clinic [102]. Even if the susceptibility tests are performed correctly, variations may occur due to discontinuous results reported at a specific interval, usually at a 2-fold scalar dilution. When variations occur, MIC values may exceed these intervals, leading to incorrect doses of antibiotics, which may be harmful to the patient [101]. For this reason, it is imperative to standardize the methodology for identifying MIC values in microbiology laboratories. The standardization process is essential for prescribing a correct treatment, controlling severe infections, and for stopping the expansion of the resistance phenomenon. Moreover, the in vitro susceptibility tests used for prescribing a treatment do not provide information about the bacteriostatic or bactericidal activity of an antibiotic [102].

The heterogeneity and increased efficiency of the resistance mechanisms of *A. baumannii* strains against almost all existing antibiotics threatens with the transition to the "post-antibiotic era", indicating the acute need to search for new therapeutic approaches. One of the challenges that hinder the success of the treatment of severe infections is the emergence of the phenomenon of heteroresistance. Heteroresistance occurs when subpopulations of isogenic bacteria exhibit lower susceptibility than the general population [103]. In *A. baumannii*, heteroresistance has been reported in antibiotics such as aminoglycosides, tobramycin, gentamicin and imipenem [104][105], but also in other antimicrobial agents such as AMPs [106]. Currently, the biggest threat is the reporting of colistin heteroresistance, which implies the existence of resistant subpopulations in a susceptible isolate (MIC ≤ 2 mg/L) by in vitro susceptibility tests [107]. In the case of severe nosocomial infections produced by heteroresistant strains in the clinic, colistin treatment may cause resistance expansion and, thus, treatment failure [108]. For detection of the phenomenon of heteroresistance, various methods are used, such as BMD (broth microdilution), E-test or PAP (population analysis profile) [107]. The use of appropriate susceptibility tests to

identify heterogeneous subpopulations is essential for the success of clinical treatment. The study by Caglan et al. highlighted differences between the results obtained after the application of BMD and E-test. Using E-test, the resistance to colistin was 4.2%, while by the BMD method, a percentage of 25.8% was obtained, analyzing the same isolates. Therefore, in the case of the E-test, a large part of the resistant strains has not been identified, which is a significant mistake in the clinic [109]. Thus, clinicians should keep in mind that although the use of gradient tests is more comfortable, the results can be confusing and can negatively influence patients' treatment.

Another problem that clinicians need to consider is the emergence of heteroresistance in patients who do not have a history of colistin treatment. However, it is more common in patients who have received treatment [110]. The emergence of heteroresistance to a range of antibiotics, including last-line antibiotics, significantly impedes the management of severe nosocomial infections caused by MDRAB strains and requires increased clinical attention to identify resistant subpopulations.

4. Conclusions

Different MDR microorganisms, among which *A. baumannii* are opportunistic pathogens, able to compete in new environments where previously only commensals or non-pathogenic microorganisms existed. The survival and persistence in nosocomial environments characterized by high antimicrobial pressure have led to the emergence of *A. baumannii* as a key pathogen, whereas a few decades ago, it caused practically no disease. The incidence of MDR and virulent clones of *A. baumannii* is also increasing worldwide, at least in these specific settings.

One of the clinic's difficult challenges is establishing the correct MIC values based on which to prescribe a correct treatment. The existence of numerous factors that influence the MIC values such as the lack of standardization of the methodology makes the success of the therapy difficult. Increased attention should be paid to factors that may influence MIC values such as patient characteristics (age, disease severity) and methods applied. In addition, identifying MIC values and MBC values can provide clinicians with additional information about the antibiotics needed to prescribe the most appropriate treatment.

The emergence of heteroresistance in some bacterial subpopulations is another challenge in the management of infections caused by *A. baumannii*.

The enormous adaptability of *A. baumannii*, as well as the very diverse mechanisms for the acquisition and transfer of AR determinants, contribute to the inefficiency of most current therapeutic strategies, determining the transition to the "post-antibiotic era" and highlighting the necessity to develop new therapeutic approaches. The latest strategies include obtaining the use of AMPs, bacteriophage therapy and CRISPR technology. Although experiments have shown the potential of these strategies in combating MDRAB, there are several challenges, such as the narrow spectrum of action, low specificity, high cytotoxicity, sensitivity to enzymatic degradation and bacterial resistance. These limitations must be addressed in future studies to develop efficient strategies for the optimal management of MDRAB infections.

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