Monoclonal Antibodies: A Promising Weapon Against the Silent Pandemic of Multidrug-Resistant Bacteria

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Abstract: The silent pandemic of antibiotic resistance, exacerbated by the COVID-19 crisis, demands innovative solutions. This review explores monoclonal antibodies (mAbs) as a promising strategy against multidrug-resistant (MDR) bacterial infections. We examine the evolution of antibacterial mAbs, from early developments to cutting-edge innovations like bispecific antibodies and antibody-antibiotic conjugates. Our analysis of ongoing clinical trials reveals both the potential and challenges of mAb therapies, offering a balanced view of their clinical impact. We discuss emerging concepts such as 'programmable' antibodies and the modulation of host microbiomes, alongside the synergistic potential of combining mAbs with other novel approaches like bacteriophage therapy. The global health implications of mAb therapies are addressed, exploring their transformative potential in resource-limited settings and innovative production methods to enhance accessibility. We critically examine developmental challenges, regulatory hurdles, and economic considerations, proposing novel frameworks to accelerate progress. Looking ahead, we envision mAbs playing a crucial role in personalized approaches to infectious diseases, tailoring treatments to individual patient profiles. This review not only summarizes the current landscape but also serves as a catalyst for future research, challenging the scientific community to reimagine the fight against MDR infections. By highlighting both achievements and obstacles, we provide a comprehensive overview of antibacterial mAbs' potential to reshape antimicrobial therapy. This work aims to inspire continued innovation in this critical field, addressing one of the most pressing health challenges of our time.

Keywords: antibiotic resistance; vaccine; monoclonal antibody; anti-bacterial infection, COVID-19

The global threat of antimicrobial resistance (AMR) has reached critical levels, presenting an unprecedented challenge to public health and economic stability worldwide. (Cassini et al., 2019; CDC, 2019). Recent data paint a stark picture: annually, AMR is responsible for an estimated 1.27 million deaths globally, with projections suggesting this could rise to 10 million by 2050 if current trends persist (O'Neill, 2014). In the United States alone, more than 2.8 million antibiotic-resistant infections occur each year, resulting in over 35,000 deaths (CDC, 2022). The COVID-19 pandemic has further exacerbated this crisis. Despite guidelines recommending against routine antibiotic use for SARS-CoV-2 infections, a comprehensive study revealed that nearly 75% of COVID-19 patients received prophylactic antibiotics, even though only 8.6% had confirmed bacterial co-infections (Langford et al., 2021). This widespread misuse of antibiotics has accelerated the development of resistance. Following the peak of the pandemic, the U.S. Centers for Disease Control and Prevention reported a alarming 15% increase in hospital-acquired antimicrobial-resistant infections (Antimicrobial Resistance, 2022). Particularly concerning is the rise in resistant strains of common pathogens, including MRSA, VRE, MDR Pseudomonas aeruginosa, and carbapenem-resistant Acinetobacter, which collectively account for a significant proportion of AMR-related deaths.

The urgency for effective infection control against antibiotic-resistant bacteria in the current era is a critical concern. As bacterial resistance becomes more prevalent and complex, global awareness of essential infection control measures is expanding, particularly within healthcare settings (Ghoneim et al., 2013). These measures encompass rigorous environmental cleaning, advanced disinfection technologies, stringent hand hygiene practices, and the prudent use of antibiotics to curb further resistance. Additionally, there is a growing emphasis on antimicrobial stewardship programs and the adoption of rapid diagnostic technologies to guide appropriate antibiotic utilization (Frost et al., 2023). Despite concerted efforts, the period from 2020 to 2023 witnessed a disappointingly low number of new antibiotic approvals, with only a few novel compounds entering the market (Butler et al., 2023). Alarmingly, the pace of drug resistance development continues to outstrip that of new drug discoveries significantly, leading to a widening gap in our antimicrobial arsenal (Batchelder et al., 2023). This trend is particularly worrisome with the emergence of pan-resistant bacterial strains that are impervious to all existing antibiotics (Kim et al., 2023a). The challenge is further exacerbated by the

economic realities of antibiotic development, with many pharmaceutical companies scaling back or discontinuing their antibiotic research programs due to low returns on investment, resulting in an innovation gap in this crucial area (Kim, et al., 2023a). Given this challenging scenario, it is imperative to explore innovative approaches beyond traditional antibiotics to combat bacterial infections. Promising alternative strategies include the development of immunotherapies like vaccines and monoclonal antibodies (mAbs) targeted at combating multidrug-resistant (MDR) bacteria (McCulloch et al., 2022; Seixas et al., 2022). These approaches offer several advantages over traditional antibiotics, including high specificity, potentially lower risk of resistance development, and the ability to leverage the host immune system's power (Elemam et al., 2021). Furthermore, recent advancements in biotechnology, such as artificial intelligence (AI)-driven antibody design and novel antibody engineering techniques, have unlocked new possibilities in this domain. By leveraging AI technologies, researchers have been able to accelerate the discovery of antibodies through the design of targeted libraries enriched for specific binding properties, thereby reducing the need for extensive experimental screening (Chungyoun and Gray, 2023).

Challenges in Developing Vaccines for Bacterial Infections

From the development of human medicines to the recent battles with COVID-19, vaccines have consistently proven to be among the most cost-effective methods for preventing infectious diseases, even in immunocompromised groups **(Antinori and Bausch-Jurken, 2023)**. Research has particularly highlighted the cost-effectiveness of vaccinating against MDR bacteria, especially in children under five and in lower-middle-income to low-income countries where the disease burden is notably high (Anderson et al., 2023; Lee et al., 2023). For instance, a study in the US found that the pneumococcal conjugate vaccine (PCV13) halved the rates of antibiotic-resistant invasive pneumococcal diseases across all age groups, from 61% to 27% **(Bajema et al., 2022)**. Additionally, typhoid conjugate vaccines (TCV) have shown substantial efficacy in curbing the spread of Salmonella typhi in impoverished regions. The WHO has systematically validated the effectiveness of TCV in controlling typhoid fever in endemic areas and supports its integration into routine vaccination programs in high-risk countries (Saha et al., 2021). As antibiotics continue to lose their effectiveness, the urgency to develop new therapeutic strategies to tackle evolving MDR bacterial strains is more pressing than ever (Stokes et al., 2019). The focus on vaccines has shifted from theoretical discussions to active investigations of their practicality in clinical settings (Jansen et al., 2018; Buchy et al., 2020). Vaccination could be a strategic response to antibiotic resistance, particularly if it significantly reduces antibiotic use in high-consumption subpopulations (Davies et al., 2021). Nevertheless, the development of vaccines for MDR bacteria faces three significant challenges: technical complexities, identification of target groups, and economic feasibility (López-Siles et al., 2020).

Initially, the technical challenge involves identifying an appropriate vaccine candidate by leveraging previous methodologies that use virulence factors, surface sugar molecules, capsules, or outer membrane proteins as antigens. This is followed by the utilization of bioinformatics to screen for proteins that exhibit potential as epitopes, focusing particularly on those that are surface-exposed and highly conserved. Moreover, translating results from animal models to human clinical trials presents significant hurdles due to variations in cytokine expression levels and differences in immunological responses between humans and rodents (Mak et al., 2014). After these extensive and resource-intensive verification steps, only a handful of candidates emerge as successful (Chiang et al., 2015; Michalik et al., 2016).

Identifying the primary target groups for vaccination is crucial. Currently, vaccinations are primarily directed towards high-risk individuals, including patients in intensive care units, those with chronic conditions, ventilator users, cancer patients, and those undergoing surgery. However, the effectiveness of preoperative vaccinations or those administered at the onset of disease is limited. Defining high-risk groups in practical terms remains a challenge, which may hinder broader vaccine promotion and utilization.

The market for vaccines against MDR bacteria is relatively small. According to the U.S. Centers for Disease Control and Prevention, around 2.8 million patients contract MDR bacterial infections annually **(CDC, 2019)**. Although this figure is substantial, the incidence rate of these infections is lower compared to other diseases, yet they present higher mortality rates. Furthermore, MDR bacterial infections associated with healthcare settings significantly increase costs due to extended stays in intensive care units and prolonged hospital admissions (Pasero et al., 2021). Despite the potential impact, the economic incentives for biopharmaceutical companies are limited. The substantial investment required for research and development, coupled with short profit margins and product lifespans, renders this sector less appealing compared to more lucrative areas such as cancer treatment.

In light of the ongoing challenge posed by MDR bacterial infections and the relentless development of new antibiotics struggling to keep pace with drug resistance, exploring alternative treatment approaches becomes crucial. One such promising method is the development of therapeutic mAbs, which may offer a more viable strategy (McConnell, 2019; López-Siles, et al., 2020). Unlike vaccines, which typically require several weeks to elicit protective immunity,

mAbs can provide immediate protection upon administration. This review aims to deliver a comprehensive examination of the role of mAbs in combating MDR bacterial infections. It will cover the mechanisms through which mAbs function, delve into ongoing research and clinical trials, and discuss the challenges and limitations that accompany mAb treatments. Additionally, this review will provide a comparative analysis of mAbs against other treatments for MDR infections, addressing the regulatory and ethical considerations involved in their use. Ultimately, the goal is to furnish insights into the future possibilities of mAb therapies in the realm of MDR bacterial infections, thereby informing and guiding further research and development efforts in this pivotal area.

Advancements in the Development of Antibacterial Monoclonal Antibodies

MAbs are homogeneous antibodies produced from a single B-cell clone, each capable of targeting a specific epitope on an antigen. Historically, despite the availability of numerous antibiotics, the cost of utilizing mAbs as a treatment option was prohibitively high, leading to slower development in this field compared to cancer and autoimmune diseases (Monserrat-Martinez et al., 2019; Lu et al., 2020). Today, propelled by advancements in precision medicine and biotechnology, the demand for mAbs in anti-infective clinical applications is increasing. To appreciate the potential of mAbs, it's important to understand the diverse roles of immunoglobulin isotypes in the immune response. Immunoglobulins, also known as antibodies, are proteins produced by B cells that play a crucial role in defending the body against pathogens. There are five major isotypes of immunoglobulins (IgD, IgE, IgM, IgA, IgG), each with distinct functions and distributions within the body (Fig. 1).

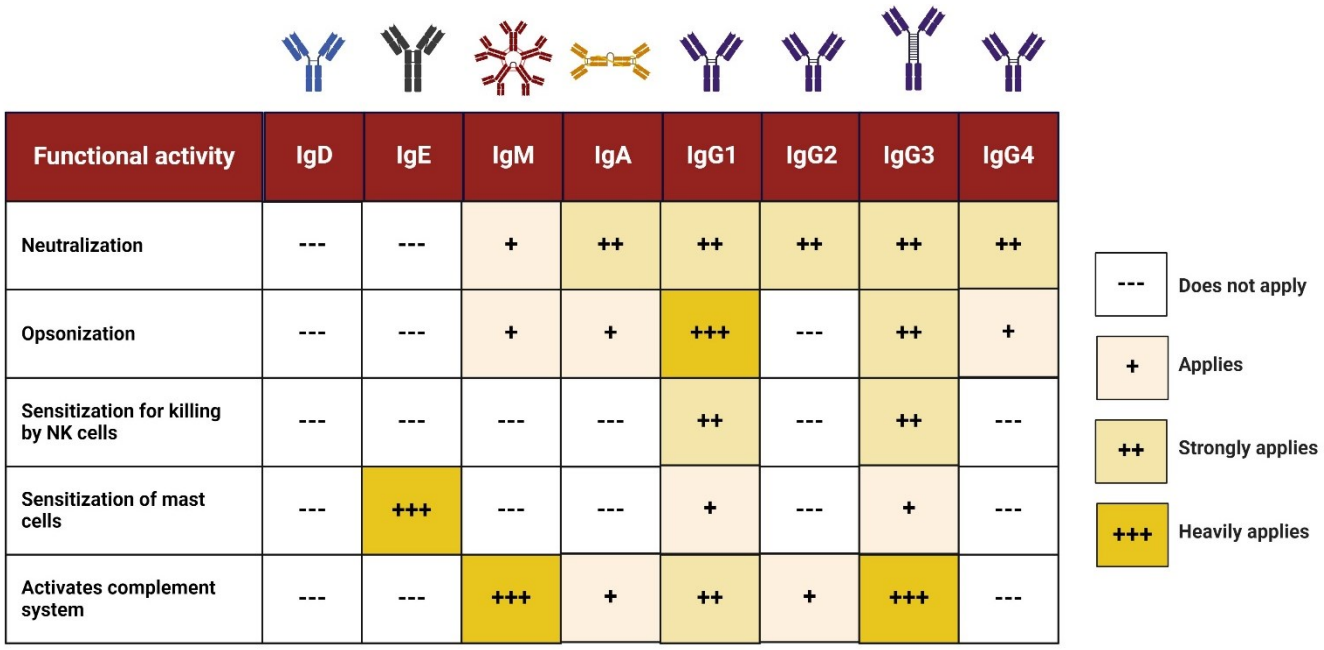


Figure 1. Functional activities of different immunoglobulin isotypes.

The table summarizes the key functions of the five major antibody isotypes (IgD, IgE, IgM, IgA, IgG) and the four subclasses of IgG (IgG1, IgG2, IgG3, IgG4) in the immune response. These functions include neutralization of pathogens, opsonization (facilitating phagocytosis), activation of natural killer (NK) cells, sensitization of mast cells (leading to degranulation and release of inflammatory mediators), and activation of the complement system (a cascade of proteins that help clear pathogens). The number of "+" symbols indicates the relative strength of each isotype's activity in a particular function, with "+++" being the highest. The "-" symbol indicates that the isotype does not perform that function.

MAbs are primarily categorized into four types based on their origin: mouse-derived, human-mouse chimeric, humanized, and fully human mAbs (Maynard, 2021) (Fig. 2). Mouse-derived mAbs, the first generation of mAb technology, are hybridomas created by fusing B lymphocytes from immunized mice with mouse myeloma cells, widely used in foundational antibody research. Human-mouse chimeric mAbs result from the genetic recombination of the mouse antibody's variable region (Fv) gene with the constant region (Fc) of the human antibody, retaining about 30%

of murine antibody characteristics (Hwang and Foote, 2005). Humanized mAbs are crafted by replacing murine mAb complementarity-determining regions (CDRs) with those in human antibodies, achieving approximately 90% humanization (Harding et al., 2010), thus maintaining the specificity of murine mAbs while being more compatible with human systems. Fully human mAbs, considered the optimal choice for therapy, are produced using techniques such as phage antibody library expression, ribosome display, and transgenic mouse technology (Klemm et al., 2021). These mAbs completely eliminate interspecies heterogeneity, reducing the risk of human anti-chimeric antibody (HACA) reactions (Hwang and Foote, 2005; Kang and Seong, 2020).

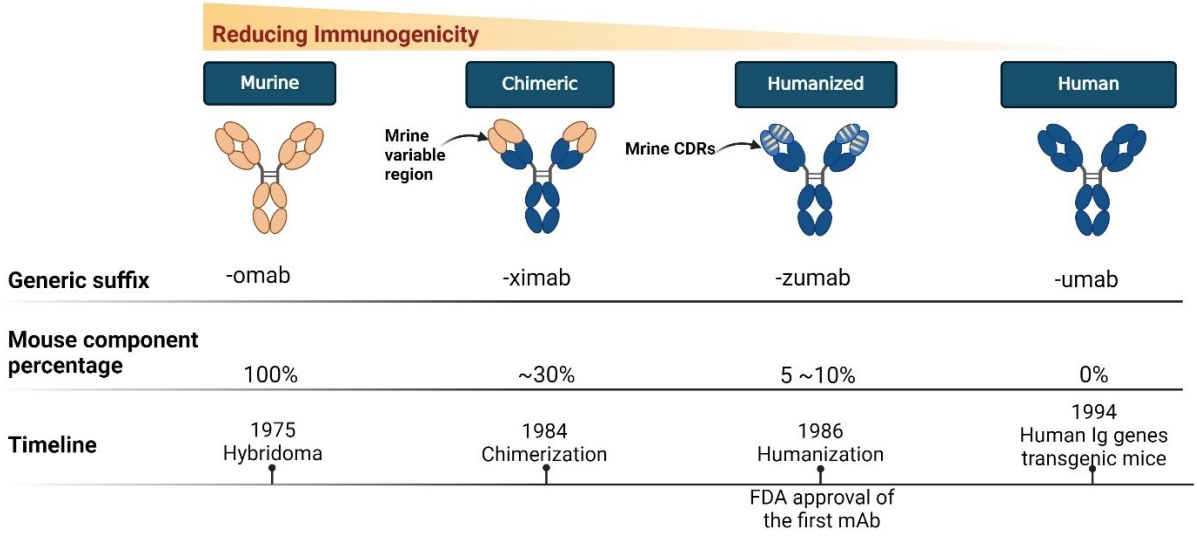


Figure 2. Progression of monoclonal antibody development

This figure delineates the evolution of mAbs, highlighting the reduction in immunogenicity from mouse to fully human mAbs. The upper bar graph illustrates the decreasing immunogenicity across four mAb generations: mouse, chimeric, humanized, and fully human mAbs. Below, three lines detail the generic name, mouse component percentage, and first approval year for each mAb type, showcasing the advancements in mAb development over time. CDRs, complementarity-determining regions.

Compared to traditional antibiotics, mAbs offer several distinct advantages for treating bacterial infections: (1) High specificity, effectively targeting MDR bacteria without affecting normal intestinal flora (Wang-Lin and Balthasar, 2018); (2) A strong safety profile; (3) The potential to be used in conjunction with standard antibiotics as antibody-drug conjugates, which can reduce dosage requirements and minimize selective pressure (Mariathasan and Tan, 2017); (4) Enhanced affinity and safety through genetic modifications, such as the development of single-chain fragment variable (scFv) antibodies and fully human antibodies (Hwang and Foote, 2005; Kang and Seong, 2020); (5) Extended half-life, ensuring sustained bioavailability and offering benefits in dosing compliance and adherence (Wang-Lin and Balthasar, 2018); (6) Vital therapeutic benefits for immunocompromised patients or those unsuitable for vaccination (Motley et al., 2019) ; (7) Environmentally friendly production processes that use minimal chemical agents (Pietrzykowski et al., 2013) ; (8) Absence of environmental accumulation, unlike antibiotics; (9) Lower likelihood of developing drug resistance, due to targeting specific virulence factors rather than essential survival proteins (Szijártó et al., 2018). In addition, mAbs are currently being explored in several clinical trials as potential adjuncts to antibiotic therapy, showing promise in enhancing treatment outcomes while aiming to reduce or replace the use of antibiotics (Zhou et al., 2016; Mariathasan and Tan, 2017; Cavaco et al., 2022).

Despite their advantages, all mAbs are recognized as foreign by the recipient's immune system, which can lead to the production of neutralizing antibodies or pathological immune responses. Chimeric mAbs inherently contain murine elements that may induce HACA responses. Similarly, humanized and fully human mAbs might trigger anti-drug antibody reactions, potentially impacting their pharmacokinetics (PK) and therapeutic efficacy (Wang-Lin and Balthasar, 2018).

Current Research and Clinical Trials

Table 1 summarizes the development of clinical trials for antibacterial mAbs as recorded on the ClinicalTrials.gov website (Medicine, 2023). From the presented summary, it is evident that the main targets for the developed antibacterial mAbs are neutralizing toxins/virulence factors (bacterial exotoxins, perforin systems), highly conserved surface carbohydrates and outer membrane proteins, as well as biofilms and iron ion capture functional factors. Upon targeting these elements, the agents subsequently block host cell bacterial invasion, reduce biofilm formation, neutralize toxins, and induce complement, with consequent opsonophagocytosis and other immunomodulatory functions that subsequently occur in immune cells (Fig. 3). Furthermore, since antibacterial mAbs are not directly bactericidal, but instead enhance the immune response against them, or attenuate bacterial pathological activity, drug resistance due to selective pressure is less likely to occur (Wang-Lin and Balthasar, 2018; Chiang et al., 2020) .

In addition to raxibacumab and obiltoxaximab, which are anti-Bacillus anthracis toxins crucial for bioterrorism defense, one of the most successful products to undergo clinical trials for bacterial infections is bezlotoxumab (Zinplava), used to treat recurrent outbreaks of *Clostridium difficile* infection [50]. In 2016, the U.S. FDA approved bezlotoxumab for use in high-risk populations aged 18 and above, including those receiving antibiotic treatments, broad-spectrum antibiotics, long-term gastric acid inhibitors, and individuals who have undergone gastrointestinal surgery [50]. Bezlotoxumab, a human IgG1 monoclonal antibody, targets toxin B of *C. difficile*, neutralizing it to reduce damage to the intestinal wall and inflammation. The success of bezlotoxumab in treating *C. difficile* has spurred further development of antibacterial mAbs (Table 1).

Additionally, mAbs can help reduce drug resistance by minimizing selection pressures and directly targeting essential outer proteins involved in resistance mechanisms. For example, mAbs can target the type 3 secretion system (T3SS) in *P. aeruginosa* or outer membrane proteins in *A. baumannii* and *Salmonella* spp [51,52]. Huang et al. demonstrated that immunization with A. baumannii outer membrane vesicles produced polyclonal antibodies that significantly enhanced the susceptibility of MDR *A. baumannii* to levofloxacin and ciprofloxacin in both in vivo and in vitro settings, by targeting several outer membrane proteins [53]. These findings highlight the potential of mAbs as a powerful tool in combating infections caused by multidrug-resistant bacteria.

Table 1. The ongoing clinical trials for anti-bacterial mAbs are accessible on ClinicalTrials.gov (accessed on 1 June 2024).

Agents	Bacterial Species	mAbs Target	Sponsor(s)	Phase of Trial	NCT number	Origin
Tefibazumab	*Staphylococcus aureus*	clumping factor A	Bristol-Myers Squibb	II	NCT00198289	Humanized
514G3		cell wall moiety Protein A (SpA)	XBiotech	II	NCT02357966	Human (isolated and cloned from a healthy human donor)
MEDI4893 (Suvratoxumab)		alpha-hemolysin	Medimmune	II	NCT02296320	Human (VelocImmune mice)
ASN-100 (ASN-1 and ASN-2)		alpha-hemolysin, gamma-hemolysin, bicomponent leucocidin (HlgAB, HlgCB, LukED, LukSF, and LukGH)	Arsansis	II	NCT02940626	Human
AR-301 (Tosatoxumab)		alpha toxin	Aridis Pharmaceuticals	III	NCT03816956	Human (convalescent patient B-cell)
DSTA-4637S		Teichoic Acid (Antibody-Antibiotic Conjugate)	Genentech and Roche	I	NCT03162250	Human
Aurograb®		ABC transporter GrfA	NeuTec Pharma / Novartis	III	NCT00217841	scFv

KB001	*Pseudomonas aeruginosa*	type III secretion system, PcrV	KaloBios	II	NCT00638365	humanized PEGylated Fab
PsAer-IgY		surface protein (Flagellin)	Mukoviszidose Institut gGmbH	III	NCT01455675	Chicken egg yolk
AR-105 (Aerucin)		alginate	Aridis Pharmaceuticals	II	NCT03027609	Human
KBPA-101 (Aermab)		LPS O-antigen (serotype O11)	Aridis (Kenta Biotech)	II	NCT00851435	Human
MEDI3902		type III secretion system, PcrV, exopolysaccharide, Psl	Medimmune	II	NCT02696902	Human (bispecific phage display and VelocImmmune mouse)
MK-3415A (actoxumab-bezlotoxumab)	*Clostridium difficile*	toxin A/B	Merck Sharp & Dohme	III	NCT01513239	Human
Bezlotoxumab (Zinplava®)		toxin B	Merck Sharp & Dohme	IV	NCT03880539	Human
GS-CDA1/ MDX-1388		toxin A/ C-terminal toxin B fragment	MassBiologics/ Merck	II	NCT00350298	human
Raxibacumab (ABthrax®/Anthrin®)	*Bacillus anthracis*	protective antigen (PA) component of anthrax toxin	Human Genome Sciences	IV	NCT02177721	Human (phage display)
Obiltoxaximab (Anthim®, ETI-204)		PA component of anthrax toxin	Elusys Therapeutics	IV	NCT03088111	Human-mouse (hybridoma)
MDX-1303 (Valortim®)		Uncleaved and cleaved PA	PharmAthene	I	NCT00964561	Human
AVP-21D9 (ThravixaTM)		PA component of anthrax toxin	Emergent BioSolutions	I	NCT01202695	Human
NTM-1632/3	*Clostridium botulinum*	botulinum neurotoxin type B	NIAID	I	NCT02779140	Humanized
XOMA 3ab		botulinum neurotoxin type B	XOMA/NIAID	I	NCT01357213	Humanized
A82 / B86	*Clostridium Tetanus*	anti-tetanus toxin	Changchun BCHT Biotechnology Co.	I	NCT06360250	Human
TRL1068	Biofilm—multiple species	biofilm scaffolding proteins DNABII	Trellis Bioscience	I	NCT04763759	Human

CMTX-101			Clarametyx Biosciences, Inc.	I	NCT05629741	Human
F598	Multiple species	Poly-N-acetylglucosamine (PNAG)	Alopexx Pharmaceuticals	II	NCT03222401	Human
Pagibaximab (BSYX-A110)	*Staphylococcal Sepsis*	lipoteichoic acid	Biosynexus	III	NCT00646399	Humanized
XJ103	*Streptococcus pneumoniae*	α-isopropylmalate dehydrogenase	Starmab biologics Co,.ltd	I	NCT06026748	humanized
cαStx1/cαStx2	Shiga Toxin-Producing *E. coli*	shiga toxins	Thallion Pharmaceuticals	II	NCT01252199	Humanized

Mechanisms of Action: How Monoclonal Antibodies Combat MDR Bacteria

mAbs offer a multifaceted approach to combating MDR bacteria, targeting conserved surface antigens such as lipopolysaccharide (LPS), capsular polysaccharides, and outer membrane proteins. This specificity allows mAbs to recognize and attach to bacterial cells effectively, even in antibiotic-resistant strains (Seixas, et al., 2022). Figure 3 illustrates the diverse mechanisms by which mAbs combat MDR bacteria:

Neutralizing bacterial toxins and virulence factors (Fig. 3A): mAbs can bind to and neutralize toxins produced by bacteria, preventing them from damaging host cells. For instance, bezlotoxumab, an mAb developed to target *Clostridioides difficile* toxin B, has shown efficacy in reducing the recurrence of *C. difficile* infections (Zurawski and McLendon, 2020a). The figure shows mAbs neutralizing and inhibiting toxins before they can interact with the bacterial cell surface. Blocking receptor binding (Fig. 3B): mAbs can interfere with bacterial adhesion to host cells by binding to bacterial adhesins or host cell receptors. This mechanism prevents the initial attachment of bacteria to host tissues, a critical step in infection. Antibody-Drug Conjugates (ADCs) (Fig. 3C): This innovative approach combines the specificity of mAbs with the potency of antibiotics. ADCs allow for targeted delivery of antibiotics to bacterial cells, potentially increasing efficacy while reducing systemic exposure and side effects. Inhibition/disruption of biofilm formation (Fig. 3D): mAbs can target key bacterial surface structures involved in biofilm formation, a critical virulence factor for many MDR pathogens. For example, an mAb targeting the Psl exopolysaccharide of *Pseudomonas aeruginosa* has been shown to disrupt biofilm formation and restrict bacterial invasion in a mouse model of pneumonia (Zurawski and McLendon, 2020a). NETosis and opsonophagocytosis (Fig. 3E): By coating the bacterial surface, mAbs mark bacteria for phagocytosis (opsonization) and destruction by immune cells. This process enhances the host's natural immune response against the pathogen (Vacca et al., 2022). Additionally, mAbs can induce NETosis, where neutrophils release extracellular traps to capture and kill bacteria. Complement-dependent cytotoxicity (Fig. 3F): mAb binding can activate the complement system, leading to direct bacterial killing via membrane attack complexes (Zurawski and McLendon, 2020a). The figure illustrates how complement proteins (C1q) bind to antibodies on the bacterial surface, leading to phagocytosis and lysis. Antibody-dependent cell-mediated cytotoxicity (ADCC) (Fig. 3G): This mechanism involves the recruitment of immune effector cells, such as natural killer (NK) cells and cytotoxic T cells, to destroy antibody-coated bacteria. The figure shows how NK cells and CD8+ T cells interact with antibody-coated infected cells, releasing cytotoxic granules to kill the target.

The ability of mAbs to employ these diverse mechanisms makes them particularly effective against MDR strains, as the targeted antigens are often essential for bacterial virulence and survival (Vacca, et al., 2022). By disrupting multiple aspects of bacterial pathogenesis, from toxin neutralization to biofilm disruption and immune system activation, mAbs offer a comprehensive approach to combating antibiotic-resistant infections.

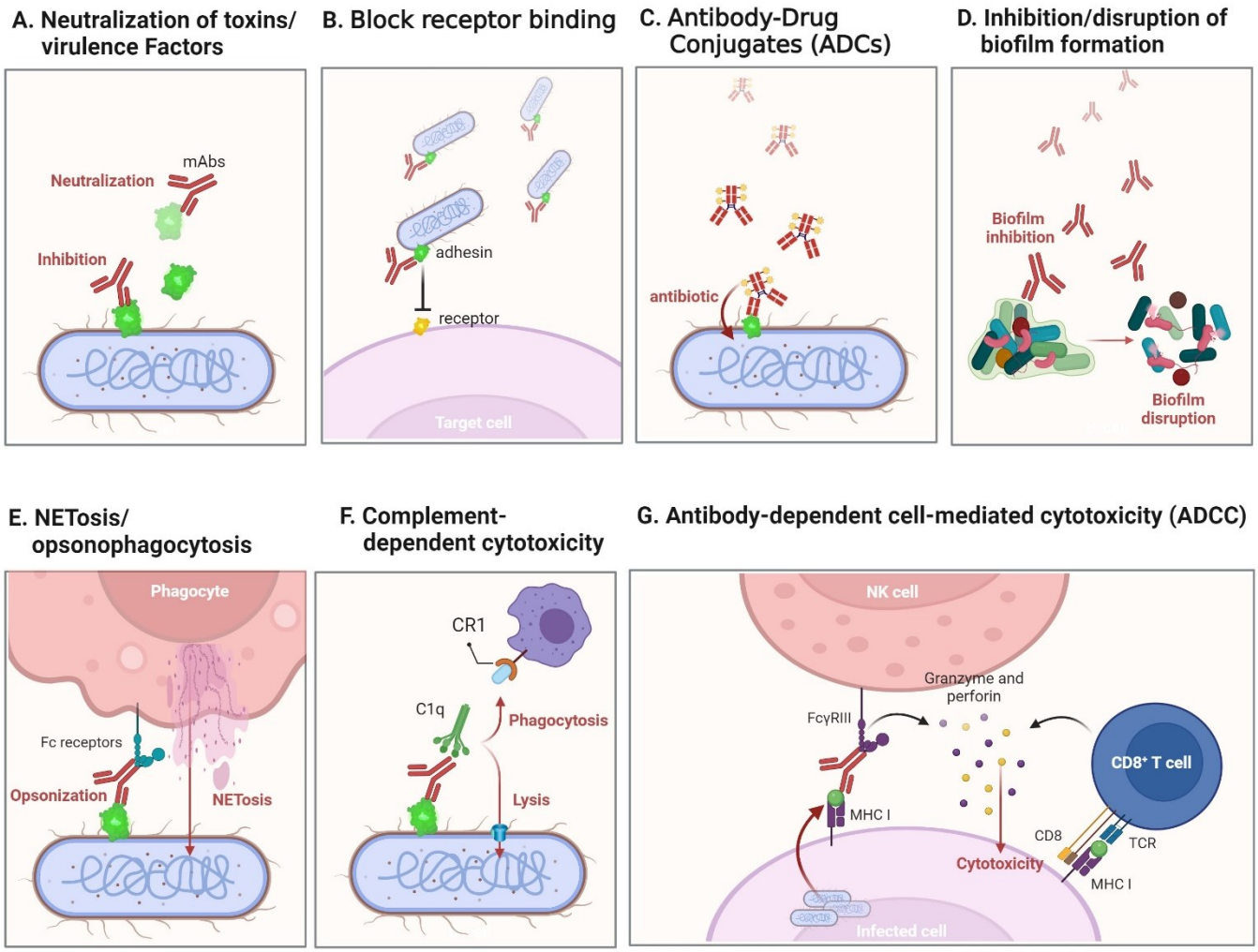


Figure 3. Mechanisms of mAb in combating bacterial infections.

This figure illustrates the intricate mechanisms through which monoclonal antibodies (mAbs) combat bacterial infections. A. Neutralization of Bacterial Virulence Factors: mAbs neutralize or inhibit bacterial virulence factors, mitigating their pathogenic effects. B. Blocking Bacterial Adhesion: mAbs block receptor-mediated adhesion, preventing bacteria from attaching to host cells and hindering infection progression. C. Antibody-Antibiotic Conjugate (ADC) Strategy: mAbs conjugated with antibiotics enhance the precision and effectiveness of targeting and eliminating bacteria. D. Disruption of Biofilm Formation: mAbs inhibit or disrupt biofilm formation, a primary bacterial defense mechanism, thereby facilitating bacterial clearance. E. mAb-Mediated NETosis/Opsonophagocytosis: mAbs promote bacterial clearance by facilitating neutrophil extracellular traps (NETs) and enhancing phagocytosis. F. Complement-Dependent Cytotoxicity: mAbs activate complement-dependent cytotoxicity, leading to the lysis of bacterial cells. G. Synergy Between Innate and Adaptive Immunity: mAbs enhance the synergy between innate and adaptive immune responses in Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC), promoting the clearance of bacterial infections (Lehar et al., 2015; Zurawski and McLendon, 2020b; Cavaco, et al., 2022; Wang et al., 2022).

Recent advancements in mAbs have shifted towards a multivalent combinatorial model, including mAb cocktails with multiple epitope binding sites. This approach encompasses neutralizing antigens more extensively, closely mimicking the human natural immune system (Gilchuk et al., 2020; Yadav et al., 2021). A recent study introduced an engineered multivalent protein biologic agent that targets five surface proteins and neutralizes five different *S. aureus* virulence factors (Buckley et al., 2023). This agent is designed to resist proteolysis, avoid Fc binding to *S. aureus* IgG-binding

proteins, and neutralize toxins. It features tandem centyrin moieties (small protein scaffolds derived from the fibronectin type III-binding domain) that bind to and neutralize two *S. aureus* leukocidins, protecting phagocytes and enhancing their antimicrobial function. Moreover, by collecting antibodies from multiple B-cell lineages, researchers have achieved varying site affinities for the same pathogen. The presence of multiple epitope binding sites offers a broader range of neutralization options and reduces the likelihood of pathogens developing escape mutations, thereby inhibiting or slowing down the development of MDR bacteria (Kennedy and Read, 2017). Multivalent antibacterial mAbs can bind to numerous antigenic sites, potentially compensating for the gaps left by antibiotics and vaccines after treatment failure (Batchelder, et al., 2023). In summary, the combined use of mAbs with antibiotic therapy presents a multi-attack approach, building evolutionary barriers against bacteria and reducing the likelihood of treatment failure due to the development of drug-resistant strains (Zurawski and McLendon, 2020a; Zurawski and McLendon, 2020b).

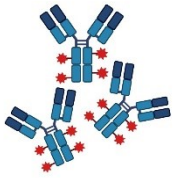
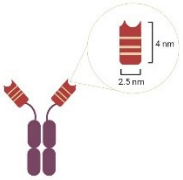
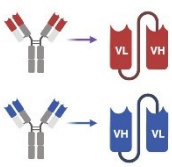
Novel Monoclonal Antibody Formats in Combatting Bacterial Infection

Several novel mAb formats have gained significant attention in recent years, including antibody-drug conjugates, bispecific mAbs, IgY, nanobodies, and single-chain variable fragments (scFv) (Table 2).

a. Antibody-Drug Conjugates: These involve drugs or toxins covalently attached to immunoglobulins, allowing for targeted delivery to specific sites. This technique shows great promise for killing cancer cells or microbes (Tvilum et al., 2023).
b. Bispecific mAbs: These combine two distinct mAbs to simultaneously target two different proteins, enabling multiple physiological or anti-tumor responses by engaging two antigens or epitopes at once (DiGiandomenico et al., 2014b).
c. IgY: This is an immunoglobulin found in birds and reptiles, showing potential applications in treating P. aeruginosa and S. aureus infections (Amro et al., 2018) (Zhen et al., 2008; Thomsen et al., 2016a).
d. These are single-domain antigen-binding fragments derived from camelid heavy-chain antibodies, offering advantages such as small size, high stability, and ease of production (Salvador et al., 2019).
e. Single-Chain Variable Fragments (scFv): These are fusion proteins of the variable regions of the heavy and light chains of immunoglobulins, while single-domain antibodies (sdAb) consist of either a light chain or heavy chain variable region (Satheeshkumar, 2020). scFv molecules have shown promise in treating neovascular age-related macular degeneration (Tadayoni et al., 2021).

Each of these novel mAb types possesses unique characteristics and advantages. Antibody-drug conjugates are particularly effective in targeting and delivering cytotoxic agents to cancer cells or pathogens. Bispecific mAbs can elicit multiple immune responses by targeting two different antigens simultaneously. IgY antibodies hold potential for specific bacterial infections. Nanobodies, due to their small size and high stability, are advantageous for various applications. scFv molecules are being explored for their efficacy in treating complex conditions like neovascular age-related macular degeneration.

Table 2. The novel types of mAbs.

Novel mAbs Types	Characteristics	Example	Advantages	Disadvantages	Ref.
Antibody-Drug conjugation 	Delivering drugs directly to specific antigens.	DSTA4637A / MRSA / Human IgG1-dmDNA31	1. Potentially minimizes the side effects associated with antibiotics. 2. Remains stable in circulation. 3. Revives antibiotics that exhibit poor pharmacokinetic properties or cause undesired host toxicity.	1. The effectiveness is limited by the abundance of antigens on bacterial cell surfaces. 2. Comprises various components with distinct chemical characteristics. 3. Presents challenges in manufacturing.	(Mariathasan and Tan, 2017) (Cavaco, et al., 2022)
Bispecific mAbs 	Simultaneously binds to two different epitopes using a mAb.	MEDI3902 / *P. aeruginosa* PcrV & Psl	1. Alternative therapeutic approach, substituting combination therapy with two monospecific drugs. 2. Sensitive immunoassays enable swift and straightforward detection of infectious diseases.	1. Production by two separate cell lines makes it costly and challenging to harvest. 2. Potential for decreased stability or susceptibility to aggregation.	(DiGiandomenico et al., 2014a) (Nyakatura et al., 2017) (Sedykh et al., 2018)
IgY 	Highly conserved to human IgG.	PsAer-IgY / *P. aeruginosa* surface protein (Flagellin)	1. Low-cost and minimal production effort. 2. Rapid production is feasible for acute illnesses. 3. Lower background of cross-reactivity.	1. Possibility of host-produced anti-IgY antibodies.. 2. Unable to activate human Fc receptors and the complement system.	(Thomsen et al., 2016b) (Lee et al., 2021)
Nanobody 	Antibody lack of light chain and constant domain.	NbD7 / *Ehrlichia chaffeensis translocated factor-1*	1. Recesses or concealed epitopes inaccessible to traditional mAbs. 2. Exhibits remarkable stability, hydrophilicity, and solubility, contributing to sustained binding capacity. 3. Diverse expression systems.	1. High uptake in kidney. 2. Risk of immunogenicity.	(Salvador, et al., 2019) (Mei et al., 2022)
scFv 	An antibody comprises only the variable regions of the heavy and light chains.	Brolucizumab / Neovascular Age-related Macular Degeneration VEGF-A	1. Preserves the original antibody's binding affinity and specificity. 2. Easily constructed, expressed, and manufactured in large quantities.	1. Limited half-life and low stability in circulation. 2. Requires high doses and continuous administration.	(Ahmad et al., 2012) (Tadayoni, et al., 2021) (Muñoz-López et al., 2022)

However, it is crucial to consider the drawbacks of these novel mAb types as well. For instance, antibody-drug conjugates may face challenges related to drug stability and off-target effects (Kim and Kim, 2015). Bispecific mAbs can be difficult to manufacture due to their complex structures (DiGiandomenico, et al., 2014b). IgY-based therapies might encounter issues with immunogenicity and purification (Amro, et al., 2018). Nanobodies may have limitations in terms of tissue penetration and immunogenicity (Salvador, et al., 2019). scFv molecules can be prone to aggregation and have shorter half-lives compared to full-length antibodies (Satheeshkumar, 2020). In summary, novel mAb types such as antibody-drug conjugates, bispecific mAbs, IgY, nanobodies, and scFv offer distinct prospects for therapeutic interventions against MDR bacteria. Each type presents its own set of advantages and disadvantages that warrant careful consideration. Further research and development efforts are needed to fully explore the potential of these novel mAb types in a post-COVID era.

Synergizing Antibacterial mAbs with Emerging Technologies

Recent advancements in cutting-edge technologies have significantly impacted the field of antibacterial mAbs, offering novel approaches to enhance their efficacy in combating bacterial infections. The integration of nanotechnology, artificial intelligence (AI), and CRISPR-Cas gene-editing systems with mAb development and application has opened up promising avenues for addressing the challenges posed by MDR bacterial pathogens. Nanotechnology has emerged as a powerful tool for augmenting the therapeutic potential of antibacterial mAbs. Engineered nanoparticles serve as effective carriers, facilitating targeted delivery of mAbs to infection sites and potentially improving their therapeutic index [76]. Furthermore, nanoparticles functionalized with mAbs can act as 'molecular traps' for bacterial toxins or whole bacteria, offering a novel approach to pathogen clearance [77]. The concept of theranostics, combining diagnostic and therapeutic capabilities, has been realized through the integration of mAbs with nanoparticles, enabling simultaneous detection and treatment of bacterial infections [78].

The development of antibacterial mAbs is being revolutionized by AI and machine learning (ML). AI has significantly impacted antibody design through various approaches, with ML models now being used to optimize critical antibody properties such as affinity, specificity, and developability (Makowski et al., 2022). These models can predict antibody characteristics using sequences or structures, allowing for the early identification of promising candidates (Kim et al., 2023b). Advanced AI-driven platforms have been developed to jointly generate antibody sequences and structures while considering the target epitope, enabling more efficient and targeted antibody design (Akbar et al., 2022). AI has also revolutionized the antibody discovery process by enabling the rapid production of antibodies through deep generative models, generating large volumes of antigen-specific data efficiently and reducing reliance on traditional experimental methods (Li et al., 2023). Moreover, AI has been instrumental in predicting immunogenicity, a crucial aspect in evaluating the safety and efficacy of antibody therapeutics, by recognizing B and T cell epitopes that may trigger an immunogenic response (Kim, et al., 2023b). The synergy between AI and other emerging technologies is further amplifying the potential of antibacterial mAbs. The combination of AI with high-throughput screening technologies and advanced structural biology techniques is enabling more rapid and accurate characterization of antibody-antigen interactions (Bennett et al., 2024). Additionally, the integration of AI with nanotechnology is opening new avenues for targeted delivery of antibacterial mAbs, potentially improving their efficacy against intracellular pathogens or biofilm-associated bacteria. As AI and ML technologies continue to evolve, we can anticipate even more sophisticated applications in antibacterial mAb development. Future directions may include AI-driven personalized antibody therapies tailored to individual patient microbiomes and immune profiles, real-time adaptation of antibody designs in response to emerging bacterial resistance patterns, and integration of AI with synthetic biology techniques for on-demand production of antibacterial mAbs. By enabling more efficient and targeted antibody development, optimizing key properties, predicting immunogenicity, and streamlining the discovery process, these technologies are accelerating our ability to combat multidrug-resistant bacterial infections (Makowski, et al., 2022). As these fields continue to advance, we can expect increasingly sophisticated and effective antibacterial mAb therapies to emerge, offering new hope in the fight against antibiotic-resistant pathogens. In the clinical domain, AI facilitates patient stratification and outcome prediction in trials of antibacterial mAbs, potentially expediting the drug development process [80].

The convergence of mAb technology with CRISPR-Cas gene-editing systems presents intriguing possibilities for targeted bacterial therapeutics. mAbs could potentially serve as delivery vehicles for CRISPR-Cas systems, enabling precise targeting of pathogenic bacteria. Furthermore, this combination might offer a means to reverse antibiotic resistance genes in bacteria, addressing one of the most pressing challenges in infectious disease management [81].

These technological integrations collectively represent a paradigm shift in the approach to antibacterial mAb development and application. By leveraging nanotechnology, AI, and gene-editing technologies, researchers and clinicians are poised to enhance the discovery, development, and therapeutic efficacy of mAbs in combating MDR bacterial

infections. This synergistic approach holds promise for overcoming current limitations and ushering in a new era of targeted, effective antibacterial therapies.

Shortcomings and Future Prospects of Antibacterial Monoclonal Antibodies

Although mAb-based immunotherapy is on the cusp of a booming research area in treating MDR bacterial infections (Table 1), several developmental challenges remain. First, despite the use of humanized mAbs, there might still be a chance of developing an HACA response, leading to therapeutic risks [31]. Second, mAb targets are typically specific to the antigens of a particular bacteria; therefore, the rapid and immediate diagnosis of pathogens will be, to a greater extent, very important. In addition, in some cases, the target antigen may only be expressed in a specific circulating strain, a single organ infection, or a disease period, which might limit the effectiveness of mAbs. For example, KB001-A targeting the T3SS protein PcrV of *P. aeruginosa*, successfully reduced the incidence of pneumonia caused by *P. aeruginosa* infection in patients using ventilators. However, it did not demonstrate the same efficacy in alleviating infection in patients with cystic fibrosis [82]. Furthermore, highly conserved outer membrane proteins, while suitable as targets, are often masked by bacterial cell surface carbohydrates, making them less accessible to mAbs. The variability in exopolysaccharide structures across serotypes poses a challenge, as a single mAb may not effectively target all variations [83]. For instance, *Streptococcus pneumoniae*, a common cause of pneumonia and meningitis, produces a capsule composed of exopolysaccharides. This capsule is crucial for the bacterium's virulence and its ability to evade the host's immune system [84]. The composition and structure of this exopolysaccharide capsule vary among different serotypes of S. pneumoniae. This variation in exopolysaccharide structures among serotypes implies that a single mAb might not provide broad protection against all serotypes of the bacterium. PNAG (β-1-6-linked poly-N-acetyl-d-glucosamine), a highly conserved exopolysaccharide found in at least 75 pathogens, is linked to pathogen survival, toxicity, and biofilm formation [85]. Owing to this, a specific IgG1 mAb (F598) was developed using deacetylated synthetic PNAG as an antigen due to its ability to mediate the killing of PNAG-expressing microbial pathogens. Despite the demonstrated efficacy of mAb F598 in mitigating microbial challenges across various models and microbes [85,86], its Phase 2 clinical trials were not pursued further. However, given its promising results in earlier phases, it holds potential for future developments.

Global Health Impact: Antibacterial mAbs in Diverse Healthcare Settings

Antibacterial mAbs have the potential to significantly impact healthcare in low- and middle-income countries by providing targeted treatment options for bacterial infections, which are often prevalent in these regions (Wang-Lin and Balthasar, 2018). The use of monoclonal antibodies as an antibacterial approach holds promise in addressing region-specific bacterial threats, offering a targeted and effective means to combat various pathogens (Zurawski and McLendon, 2020a). One of the key challenges in the global distribution and access to antibacterial mAbs lies in ensuring equitable availability and affordability, especially in resource-limited settings. Overcoming logistical and financial barriers is crucial to ensure widespread access to these life-saving treatments (Johnson et al., 2023). Future directions in the field of antibacterial mAbs may involve further research into novel antibody-peptide combinations for targeting specific bacterial strains, as well as exploring innovative strategies to enhance the efficacy and accessibility of these treatments globally. Breakthroughs in antibody development and antimicrobial peptide fusion could pave the way for more effective and accessible antibacterial therapies in diverse healthcare settings (Johnson, et al., 2023). Similar to the problems observed for vaccines and antibiotics, commercial investment tends to be influenced by market size of the disease. Therefore, the development of broad-spectrum mAbs needs to be guided by the policies of government regulators to reduce commercial barriers and enable related fields to blossom. This remains an open question that requires addressing in the foreseeable future.

Conclusion

The journey of mAbs in combating MDR bacterial infections stands at a critical juncture. As we've explored throughout this review, the potential of mAbs to revolutionize our approach to treating resistant infections is substantial, yet not without challenges. The landscape of antibacterial mAbs is rapidly evolving. From the early days of mouse-derived antibodies to the current era of fully human and engineered antibodies, we've witnessed remarkable progress. The success stories, such as bezlotoxumab for C. difficile infections, provide a glimpse of what's possible. However, the road ahead demands innovation, persistence, and a willingness to challenge conventional therapeutic paradigms.

One intriguing avenue for future research lies in the development of 'smart' mAbs capable of distinguishing between commensal and pathogenic bacteria. Such precision could revolutionize treatment, minimizing disruption to the

beneficial microbiome while effectively targeting harmful pathogens. Imagine antibodies that not only neutralize bacteria but also modulate the host immune response, enhancing natural defenses against infection. The potential synergy between mAbs and other emerging technologies is another frontier ripe for exploration. Could we see nanoparticle-mAb conjugates that more effectively penetrate biofilms? Or mAbs engineered to carry CRISPR-Cas payloads, capable of editing bacterial genomes to reverse antibiotic resistance? These may seem like science fiction today, but so did many of our current medical marvels just a few decades ago. As we look to the future, the global health implications of mAb therapies cannot be overstated. Their potential to address region-specific bacterial threats could transform healthcare in resource-limited settings. However, realizing this potential will require overcoming significant hurdles in production, distribution, and affordability. Innovative approaches, such as plant-based antibody production or the development of thermostable formulations, could be game-changers in expanding access. The road ahead for antibacterial mAbs is not without obstacles. Regulatory frameworks must evolve to keep pace with technological advancements. The economic realities of drug development continue to pose challenges, particularly for therapies targeting fewer common pathogens. Collaboration between academia, industry, and government agencies will be crucial in overcoming these hurdles. Moreover, as we delve deeper into the complexities of host-pathogen interactions, our understanding of how to best deploy mAb therapies will undoubtedly evolve. Could personalized approaches, tailored to an individual's microbiome or immune profile, become the norm? Might we see combination therapies that pair mAbs with traditional antibiotics or other novel antimicrobials, creating synergistic effects that overcome resistance mechanisms?

In conclusion, while the challenges are significant, the potential rewards of advancing mAb therapies for MDR bacterial infections are immense. As researchers, clinicians, and policymakers, we stand at the threshold of a new era in infectious disease treatment. By embracing innovation, fostering collaboration, and maintaining a patient-centered focus, we can work towards a future where MDR infections no longer pose the existential threat they do today.

The journey of antibacterial mAbs is far from over; in many ways, it's just beginning. As we move forward, let us approach this challenge with the urgency it demands, the creativity it requires, and the hope it inspires. The next chapter in the story of antibacterial mAbs is ours to write, and its impact could reshape the landscape of global health for generations to come.

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