

ANTIMICROBIAL RESISTANCE AND UPCOMING DNA VACCINE - A NEW CONCERN

Abstract

The emergence and proliferation of drug-resistant bacteria, featuring novel resistance mechanisms leading to antimicrobial resistance, continue to pose a threat to our ability to effectively combat common illnesses. A major public health concern is antimicrobial resistance, particularly in developing countries where easy access to and greater use of pharmaceuticals have led to a disproportionately higher rate of inappropriate antibiotic use and higher levels of resistance than in developed nations. When bacteria, viruses, fungi, and parasites evolve to the point where they are resistant to antibiotics, it is known as antimicrobial resistance (AMR). This increases the risk of serious medical disorders, mortality, and difficulty treating infections. Given these complications, it emphasizes the need to explore additional means of protection against well-known pathogenic microorganisms. Vaccine can be called a best alternative to provide an strong immunotherapy against bacterial and viral species. Although DNA vaccine is found to be more give more effective immune thearapy as it induce and activate both humoral and cell mediated arms of immune system in animals and huamns. This review paper dispense a well gone through idea for controlling infectious disease by strengthening immunity system of living body prior to the attack of infectious agent, its delivery into the body, its clinical uses, and its other applications against pathogenic bacterial and viral agents. With the assist of this article it can be clearly understood that why DNA vaccine is more adventitious over other well known vaccine varieties.

Keywords: Anmicrobial resistance species, DNA vaccine, Immunity

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I. INTRODUCTION

Antimicrobials are a class of medications employed for the prevention and treatment of infections in humans, animals, and plants. This category encompasses antibiotics, antivirals, antifungals, and antiparasitic drugs. The issue of antimicrobial resistance constitutes a significant public health concern, particularly in developing nations, where the ready availability and increased utilization of medications have led to a disproportionately higher prevalence of inappropriate antibiotic utilization and elevated resistance levels compared to developed countries (1). When bacteria, viruses, fungi, and parasites evolve to the point where they are resistant to drugs, this is known as antimicrobial resistance, or AMR. This increases the danger of mortality, serious sickness, and the spread of disease, and makes controlling infections more challenging. Drug resistance causes antibiotics and other antimicrobial medicines to lose their effectiveness, making the treatment of infections more challenging or perhaps unachievable (2).

Increasing antibiotic resistance species of bacterial pathogen is a enormous issue for medicine world. Therefore to get protected form mutated bacterial species carrying antibiotic resistance property an alternative means of medicine is required (3). Other alternative way of control infectious disease causing microbes or virus is vaccine. Besides of existing vaccine strategies, DNA vaccine is a newly known and designed, variety of vaccination strategy in which plasmid DNA encoding antigenic protein is injected directly into the muscle cells of the recipient and muscle cells take extracellular DNA into cell. Plasmid DNA may or may not integrated in the genome of the recipients cell, plasmid DNA has its own origin of replication, capping and polyadenine tail, some also has intron sequence so that it can easily get ready for transcription and translation and also post translation modification. After gaining entry in to the cell DNA vaccine immediately show transcription to make mRNA copies. mRNA translates to form antigenic protein which are able to trigger immune system both humoral and cell mediated immune response (4).

II. ANTIMICROBIAL RESISTANCE A GLOBAL CONCERN

Antimicrobial resistance, or the growth and spread of drug-resistant bacteria with novel resistance mechanisms, continues to pose a threat to our capacity to treat common infections. A development that is especially worrisome is the quick spread of multi- and pan-resistant bacteria throughout the world (sometimes referred to as "superbugs"), which cause illnesses that are resistant to most antimicrobial drugs now available. There aren't enough novel antibiotics undergoing clinical testing. Of the 32 antibiotics that the World Health Organization (WHO) recognized as being under clinical development in 2019, only six were considered novel. These medications target pathogens on the WHO priority list. Another significant issue is the lack of access to high-quality antimicrobials. Antibiotic shortages are having a detrimental effect on many spheres of development, particularly health care systems.

Antibiotic resistance is spreading around the world, making antibiotics less effective, leading to more deadly diseases and illnesses that are harder to cure. For example, the treatment of carbapenem-resistant gram-negative bacterial infections, a priority disease identified by the WHO, requires an urgent need for novel antibacterials. However, if current antibiotic usage continues, these novel medications will ultimately meet the same fate as their predecessors and become ineffective. Because AMR necessitates longer hospital stays and

more costly, intensive treatment, it lowers the productivity of patients and caregivers, resulting in substantial costs to national economies and health systems. The amount of people who need treatment or pass away from infections will increase in the absence of effective strategies for stopping and treating drug-resistant illnesses as well as improved access to both new and existing quality-assured antimicrobials. Riskier procedures include organ transplants, cancer chemotherapy, and surgeries like hip replacements and cesarean deliveries.

Causes of Antimicrobial Resistance (AMR): AMR develops gradually over time primarily due to genetic mutations. Microbes that are resistant to antibiotics can be found in food, plants, animals, people, and the environment (including soil, water, and air). These resistant microorganisms can spread from person to person or between humans and animals, especially when food products obtained from animals are consumed. The primary causes of antimicrobial resistance include the overuse and misuse of antibiotics, inadequate infection and disease prevention and control practices in hospitals and farms, poor access to clean water, sanitation, and hygiene (WASH) for humans and animals, restricted access to high-quality, reasonably priced medications, vaccines, and diagnostics, a lack of awareness and knowledge, and insufficient legislative enforcement.

III. DRUG RESISTANCE IN BACTERIA

Globally, there is a concerning prevalence of high resistance rates to commonly used antibiotics for treating common bacterial infections such as urinary tract infections, sepsis, sexually transmitted infections, and specific types of diarrhea. This trend indicates a diminishing pool of effective antibiotics. For instance, in countries contributing data to the Global Antimicrobial Resistance and Use Surveillance System (GLASS), resistance to ciprofloxacin, a routinely used antibiotic for urinary tract infections, ranged from 8.4% to 92.9% for *Escherichia coli* and from 4.1% to 79.4% for *Klebsiella pneumoniae*. Common gut bacterium *Klebsiella pneumoniae* has become resistant to carbapenems, which are last-resort antibiotics. This bacteria plays a major role in hospital-acquired infections, such as bloodstream infections, pneumonia, infections in neonates, and infections in intensive care units. Due to resistance, more than half of patients in some countries receiving treatment for *K. pneumoniae* infections do not respond to carbapenem medications.

Fluoroquinolone medications, commonly used for treating urinary tract infections, face widespread resistance in *Escherichia coli*. In numerous countries worldwide, more than half of patients receiving these medications no longer respond effectively.

Life-threatening infections caused by Enterobacteriaceae, such as *E. coli* and *Klebsiella*, that are resistant to carbapenems can only be treated as a last resort with colistin. Furthermore, colistin-resistant bacteria that cause diseases for which there is presently no effective antibiotic treatment have been found in a number of nations and areas.

Staphylococcus aureus, a bacterium commonly found on the skin, frequently causes infections in both the general population and healthcare settings. Compared to infections that heal, infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) increase the chance of death by 64%.

A new AMR indicator was added to the Sustainable Development Goal (SDG) monitoring framework in 2019. This indicator monitors two types of drug-resistant bacteria: *E. coli* resistant to third-generation cephalosporins (3GC) and methicillin-resistant *Staphylococcus aureus* (MRSA). 25 nations, territories, and regions reported data on MRSA-caused bloodstream infections to GLASS in 2019, whereas 49 countries contributed data on *E. coli*-caused bloodstream infections. Although these results are not yet nationally representative, the median rate for methicillin-resistant *S. aureus* was 12.11% (IQR 6.4-26.4) and for *E. coli* resistant to third-generation cephalosporins was 36.0% (IQR 15.2-63.0).

A major obstacle to the management and control of gonorrhoea has been the extensive resistance found in *Neisseria gonorrhoeae* strains that are exceedingly varied. Resistance to fluoroquinolones, tetracyclines, macrolides, sulphonamides, penicillins, and early-generation cephalosporins has increased significantly. The majority of countries currently exclusively offer injectable extended-spectrum cephalosporin (ESC) ceftriaxone as an empirical monotherapy for gonorrhoea.

- 1. Drug Resistance in Mycobacterium Tuberculosis:** Antibiotic-resistant: The global tuberculosis epidemic is at risk from newly emerging *Mycobacterium tuberculosis* strains. WHO estimates that more than 500,000 new cases of rifampicin-resistant tuberculosis (RR-TB) were reported globally in 2018. Two of the most effective anti-TB drugs do not treat multi-drug resistant tuberculosis (MDR-TB), which accounts for the bulk of these cases. Out of the approximately 500,000 people who contracted MDR/RR-TB in 2018, just one-third of the cases were found and reported. The duration, efficacy, and cost of MDR-TB treatment regimens are higher than those for non-resistant TB. Approximately 60% of people with MDR/RR-TB who get treatment do not fully recover. A major worry is resistance developing to new "last resort" TB medications used to treat drug-resistant TB. It was estimated that 3.4% of newly diagnosed cases of tuberculosis and 18% of those that had received treatment in 2018 were MDR/RR-TB cases.
- 2. Drug Resistance in Viruses:** Immunocompromised patient populations are increasingly concerned about antiviral treatment resistance due to the selection of resistant virus strains by prolonged drug exposure and persistent viral replication. Antivirals, particularly antiretroviral (ARV) drugs, have become resistant to most of them.

The emergence of HIV drug resistance (HIVDR) poses a risk to the effectiveness of all antiretroviral (ARV) medications, including newer drug classes. HIVDR can develop in individuals receiving ARV treatment, and individuals can acquire HIV strains that are already resistant to drugs. Pretreatment HIV drug resistance (PDR) to In the majority of the nations in Africa, Asia, and Latin America where data were followed, the percentage of patients using non-nucleoside reverse transcriptase inhibitors (NNRTIs) as first-line therapy exceeded 10%. In sub-Saharan Africa, about 50% of newborns newly diagnosed with HIV have a virus resistant to non-neonatal retroviral therapy (NNRTI). PDR is a worryingly common condition in young infants. The use of the novel drug dolutegravir as the recommended first-line treatment for both adults and children has been encouraged by these findings, according to the most recent WHO ARV guidelines. It is essential to administer this drug as soon as possible in order to lessen the negative effects of NNRTI resistance.

The considerable cost difference between first- and second-line treatment regimens and rising resistance levels has serious economic ramifications. The World Health Organization's HIV medication resistance program keeps track of the emergence and spread of resistance to both older and newer HIV therapies worldwide.

3. **Drug Resistance in Malaria Parasites:** Drug-resistant parasites are becoming more common, which raises the risk of malaria control and increases morbidity and mortality rates. As the first line of treatment for non-complex *P. falciparum* malaria, artemisinin-based combination treatments (ACTs) are used in the majority of countries where there is an epidemic of the disease. ACTs consist of a companion medication in addition to artemisinin. Research conducted in Cambodia, Lao People's Democratic Republic, Myanmar, Thailand, and Vietnam between 2001 and 2019 confirmed that these countries had partial resistance to artemisinin and resistance to numerous ACT partner drugs. This increases the difficulty of determining the optimal course of treatment and demands close observation. A new body of African research has revealed evidence that partial artemisinin resistance is caused by mutations that occur in Rwanda. The ACTs that have been tested are still quite successful. However, increased resistance to artemisinin and the ACT companion medications presents a significant risk to public health and may undermine significant advancements achieved in the fight against malaria.
4. **Drug Resistance in Fungi:** Drug-resistant fungal infections are becoming more common, which makes the already complicated treatment environment worse. Challenges in treating numerous fungi have emerged, particularly regarding issues of toxicity, especially among individuals with underlying conditions such as HIV. With more and more reports of drug-resistant *Candida auris*, one of the most common invasive fungal diseases, emerging resistance to caspofungin, amphotericin B, fluconazole, and voriconazole. This condition results in more challenging-to-treat fungal infections, which raises the cost of treatment alternatives and leads to treatment failures and extended hospital admissions. In addition to evaluating the pipeline for the development of antifungal medications, the World Health Organization (WHO) is undertaking a thorough worldwide assessment of fungal illnesses and will issue a list of important fungal pathogens for public health consideration (2).

IV. ADVANTAGES OF DNA VACCINE OVER OTHER VACCINE AND AN ALTERNATIVE FOR ANTI-MICROBIALS

However, DNA vaccine was designed by Enzo Paoletti and Dennis Panicali at New York in 1983, from then till now many researchers are performed experiments on DNA vaccine some are successful and few not as its tests on human body needs many experimental trials on the genipig animals. DNA vaccines are superior than current vaccinations in a number of ways. DNA vaccines induce both humoral and cell-mediated immunity; (ii) the encoded protein from plasmid DNA expressed in its natural form, i.e., no denaturation and alteration; and (iii) DNA vaccines promote sustained expression of antigen. (iv) Produces a notable memory for immunity. (v) The DNA vaccine can be stored without refrigeration (4). The test hypothesis that a synthetic DNA vaccine encoding HPV immunogens for the development of nuclear oncogenes E6 and E7 from HPV types 16 and 18 (two high risk genotypes) would induce cytotoxic T-lymphocytes that may be impact HPV-induced cervical intraepithelial neoplasia (CIN) was advanced by reports mentioning a first-in-human (FIH)

clinical trial. The vaccine was primarily known to be highly immunogenic, eliciting antibodies and cytotoxic T cells in nearly all inoculated participants, according to an early immunogenicity study [5] (6).

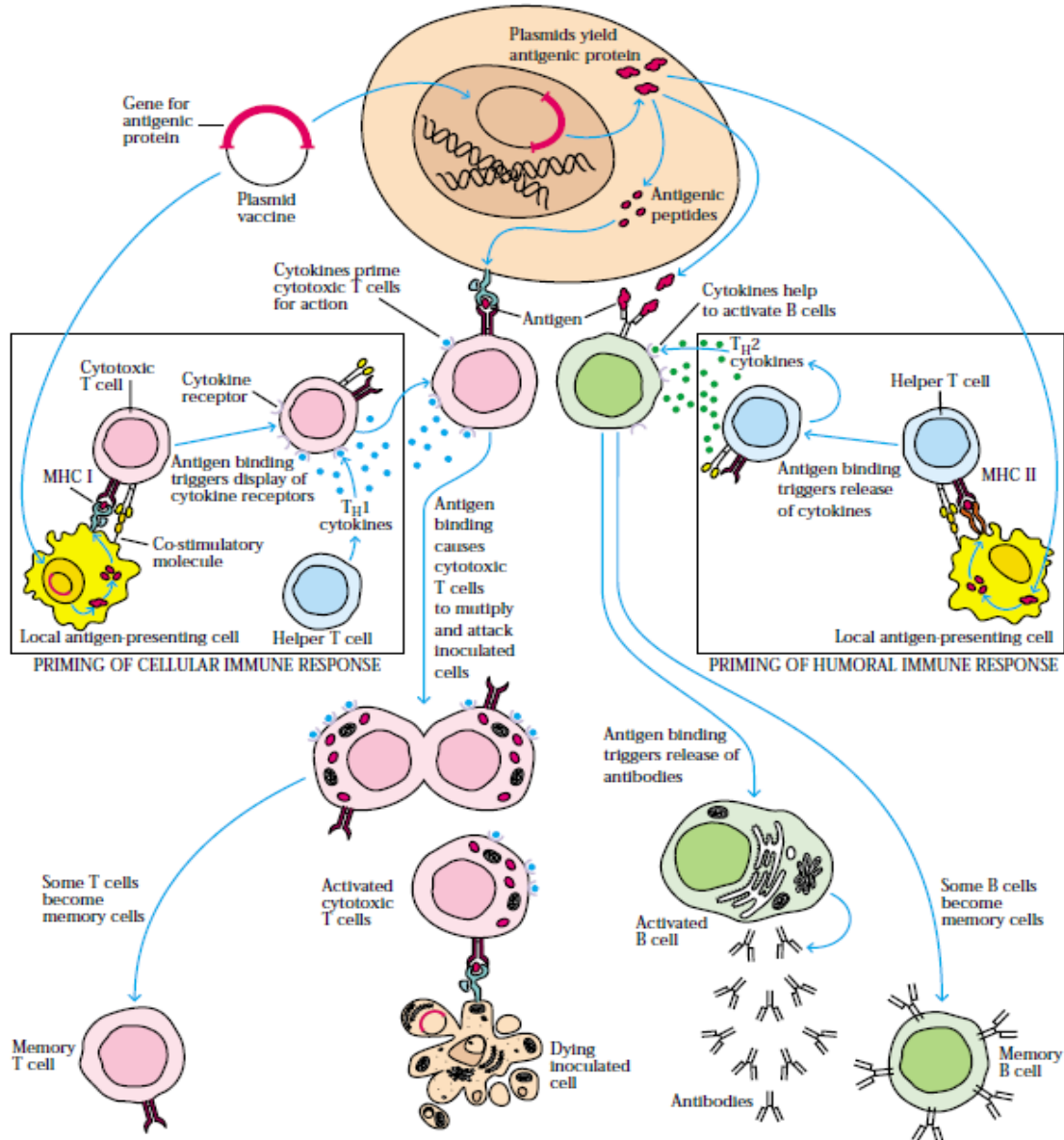


Figure 1: Use of DNA vaccine rises both humoral and cell mediated immunity. Injected gene is expressed in muscles and in nearby APC cell. The peptides translated from cells expressed on the surface by processing as an endogenous antigen by MHC class I and therefore activate cytotoxic T lymphocytes. Some protein expressed as soluble secreted protein which is taken up by APCs, processed and expressed by MHC II to the B lymphocytes and activate humoral branch of immunity. (Source:4)

1. DNA Vaccine Delivery: According to a plethora of studies, synDNA facilitates the production of humoral and cellular responses to pathogen antigens incorporated into the cell, which have an effect on challenge model systems. Intramuscular, intradermal, and subcutaneous injections are the three primary classical methods of vaccine distribution.

These methods primarily target local myocytes [7], keratinocytes [8], and antigen-presenting cells (APC) that are located close to the injection site [9]. On the other hand, it has been found that high concentration formulations used in intramuscular (IM) inoculation and, more recently, intradermal (ID) delivery can generate sustained immunity. Numerous *in vivo* delivery techniques, such as electroporation, jet delivery, gene gun administration, and nanoparticle distribution, can improve DNA uptake. Adaptive electroporation (EP) enhances transformation efficiency in the syringe and needle distribution process by managing the energy supplied during *in vivo* EP. For the immunization, CELLECTRA-EP and intramuscular injection were utilized. Ratioconversion was noted in 95% of cases. Sixty-six percent of patients still maintained MERS-CoV-specific T cell responses sixty days following their final dose (10)(11).

The transfected macrophages and dendritic cells that are local antigen-presenting cells (APCs) can travel directly to the localized lymph node (LN) and convert naive B cells into plasma cells, which is essential for starting the immune response (12). Local cells where the vaccine is given exhibit exocytosis or death after the antigenic DNA is translated, translated, and shed exogenously. APCs then pick up the local cells for cross-presentation. Shedded exogenous soluble antigen can be immediately drained by wandering B cells and neutrophils, who then carry it to the extracellular spaces in the surrounding environment and the local regional lymph node, where it can activate B cell immunity. When antigen is presented on major histocompatibility complex-1 (MHC I) or MHC II molecules, local tissue functions as a protein factory that presents the antigen, allowing lymph nodes to expand and activating CD8⁺ primed T cells (also known as cytotoxic T lymphocytes, or CTLs) and CD4⁺ T cells, respectively (13).

- 2. DNA Vaccine on Viral Diseases:** A DNA vaccine has demonstrated its effectiveness against well-known infectious viruses. It is expected that more than 2 million new cases of HIV-1 infections happen each year. Furthermore, considering the recent global COVID-19 pandemic, which has resulted in millions of deaths worldwide, the identification of the SARS-CoV virus's structure and genome has been crucial for vaccine development. Antibodies are predominantly produced against the N and S proteins found on the viral envelope, according to research (6)(14). The N protein, which carries the viral genome and facilitates the release of virus particles from cells, and the S protein, which interacts to host cells via its receptor-binding domain and initiates an infection in host cells, are particularly important for pathogenesis (15).

To stop the COVID-19 virus from spreading, a DNA vaccine must be created. All DNA vaccines that are presently undergoing clinical trials, including AG0301-COVID19 (ClinicalTrials.gov number, NCT04463472), have used the S protein as the antigen. The vaccination schedule for this vaccine consists of two injections, given intramuscularly two weeks apart at a low dose of 1.0 mg and a high dose of 2.0 mg (16). Currently, healthy volunteers between the ages of 20 and 65 are being sought after in order to evaluate the immunogenicity of the vaccination (16).

DNA vaccines have also shown promise as potential immunotherapeutic agents against cancer. It is simple to construct and modify DNA plasmids nowadays in order to elicit powerful cell-mediated immune responses. Prospectively, DNA vaccines ought to endeavor to augment anti-tumor immunity through the advancement of immunological

tolerance, disturbance of immune-suppressive networks inherent in the tumor microenvironment, and generation of enduring memory (17). These DNA vaccines have proven effective against various well-known disease-causing viruses such as HIV, Zika, Ebola, Dengue, Chikungunya, Herpes, and coronaviruses, among others. The increasing diversity of viral genomes and the structural variations among existing viral species underscore the pressing need for an effective prophylactic vaccine.

- DNA Vaccine against Bacterial Disease:** There is a pressing need for the development of newly designed vaccines to combat antibiotic-resistant bacterial species and to enhance our ability to manage bacterial diseases (18). It is worth noting that among the licensed vaccines currently available, targeting nearly nine bacterial pathogens, none of them are based on gene vaccine technology, specifically DNA vaccines, as indicated by records from the FDA. The rates of both mortality and morbidity underscore the necessity for novel approaches, irrespective of the utilization of existing bacterial vaccines. Various methods for eliciting an immune response against bacteria have been explored, and it has been observed that these approaches may not be highly effective (3). Below is a table illustrating certain bacterial diseases and their control strategies through DNA vaccines:

Table 1: Pathogenic Bacterial Species and their Antigenic Protein (source: 3)

Bacteria	Disease causes	Immune response required	Antigen shows immunogenicity
Mycobacterium tuberculosis	Tuberculosis	CTL	Ag85A, Ag85B, FbpA, Htpx, Rv3407, Hsp65, Mtb75F, ESAT-6, MPT64
Streptococcus pneumoniae	Pneumoniae	Anti-capsular polysaccharide antibodies	PspA
Streptococcus group A	Throat and nostrils infection	Anti-capsular polysaccharide, protein and exotoxin antibodies	HtpA
Haemophilus influenzae type B	Pneumonia, Septicaemia, meningitis etc	Anti-capsular polysaccharide antibodies	
ETEC	Diarrhea	Anti-surface protein antibodies	Ec-FA
Clostridium tetani	Tetanus	Anti-toxin antibodies	TetC
Clostridium botulinum	Botulinism	Anti-exotoxin antibodies	AHc
Salmonella typhi	Typhoid	CTL	OmpC
Neisseria meningitidis	Meningitis	Anti-surface protein and capsular polysaccharide antibodies	PorA, NMB0088, MenB C

Vibrio cholerae	Cholera	Anti-toxin antibodies	
Helicobacter pylor	Gestritis or peptic ulcer	Anti-surface protein antibodies	HP-NAP, Ure-B, HP-kat, HspA, HspB
Streptococcus group B	Infection in throat and tonsils	Anti-capsular polysaccharide antibodies	LrrG
Staphylococcus	Skin infection	Anti-capsular polysaccharide antibodies, proteins and CTL	PBP2a, pCl, pClfa, pSrt
Chlamydia pneumoniae	Pneumonia	CTL	MOMP, Omp2
Mycoplasma pulmonis	Infection in pulmonis	CTL	A7-1, A8-1
Borrelia burgdorferi	Lyme disease	Anti-surface protein antibodies	OspA, OspC
Pseudomonas aeruginosa	Malignant external otitis, endophthalmitis, endocarditis	Anti-surface protein antibodies	OprF, FliC R90A
Bacillus anthracis	Anthrax	Anti-exotoxin antibodies	PA
Listeria monocytogenes	Listeriosis		Listeriolysin O, p60
Chlamydia trachomatis	Chlamydia	CTL	pORF5

4. DNA Vaccine Development and Clinical use in Medicine: Conventional protein/peptide-based vaccines and attenuated vaccine are intended to induce antigen-specific adaptive immune specifically humoral responses but may be some time failed and reversion of attenuated vaccine viral may cause critical infection.

In contrast to all of this, DNA vaccination is affordable, simple to produce, and safe to handle. Vaccines based on DNA are more stable. Furthermore, a wide range of uses for DNA vaccines are being researched, including the treatment of infectious, autoimmune, cancer, and allergy disorders. It is reported that more than 500 clinical trials focusing on DNA vaccination have been registered in the US, with a particular focus on viral infections and cancer. The growing number of DNA vaccines being evaluated in clinical trials indicates how crucial they will be for advancements in medicine.

V. CONCLUSION

One of the main concerns is the significant morbidity and mortality that come with bacterial infections due to antibiotic resistance. Multi-drug resistant bacteria, fungi, viruses, parasites, and both types of negative and positive gram-positive bacteria can be difficult to treat and even resistant to common drugs. Bacterial infections and the diseases they produce provide issues that necessitate the development of novel therapeutic options and alternative

antimicrobial drugs because there are currently no viable therapies, prevention measures, or new antibiotics. The DNA vaccination has emerged as one of the most exciting uses for gene therapy at the moment. When the idea was originally proven in the early 1990s, it drew a lot of attention due to its distinct capacity to quickly trigger humoral as well as cellular immune responses. This paper tried to discuss about the upcoming concern of DNA vaccine as a alternatives of antimicrobial resistance. Given that DNA vaccines have proven to be successful in eliciting immunological responses, future developments are worth considering. Therefore, in this connected subject of immunology, public health, and microbiology, additional research of this kind is required.

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