The Molecular Pathways of Diphtheria Toxin: Potential Consequences for Drug Design

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Abstract

Diphtheria, triggered by *Corynebacterium diphtheriae*, is a disease characterized by a pseudomembrane. It affects the pulmonary system, causing serious effects, and may appear as inactive, respiratory, or cutaneous. Vaccination has improved its global impact, although it remains prevalent throughout multiple nations that are underdeveloped. *Corynebacterium diphtheria* produces the diphtheria toxin (DT), which has three functional domains. It traverses cells and hinders protein synthesis by disabling elongation factor 2 (EF-2). Current diphtheria treatments include antitoxin medications, antibiotics, and immunization techniques. Antitoxin therapy obtained from immunized animals encounters obstacles such as serum sickness, motivating the hunt for recombinant human antibodies. Penicillin and erythromycin are examples of antibiotics that are employed to treat infections and stop their spread. Despite coverage deficiencies, vaccination strategies such as the Tdap as well as the Td vaccines are essential for preventing diphtheria. Targeting toxin mechanisms, developing toxin inhibitors, and investigating new antitoxin possibilities are the main goals of developing new therapeutic approaches for diphtheria. Monoclonal antibodies, antimicrobial peptides, and disulfiram are examples of possible therapies. Enhancing adjuvants and combination vaccines to boost immune responses is another goal of vaccine development. The necessity for substitutes, such as monoclonal antibodies, is highlighted by difficulties with the production of antitoxin antibodies against diphtheria (DAT). Small-molecule inhibitors, specifically those that target Poly (ADP-ribose) polymerases (PARPs) and mono-ADP-ribosyltransferases, are the main focus of current diphtheria research. As bacteria develop resistance to several drugs, antimicrobial resistance is growing, increasing morbidity, mortality, and medical expenses. Drug inactivation, target modification, active efflux, and drug uptake limitation are examples of resistance mechanisms. There are still issues with vaccine safety and effectiveness, particularly for certain patient populations.

Keywords: Bacteria, Diphtheria, Toxins, Molecular pathways, Drug delivery

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Introduction

Because of the properties of the pseudomembrane that the organism itself produces over the colonization site, the term "diphtheria" stems from the Greek word "diphtheria," which means "hide or leather" (Chaudhary & Pandey, 2020). *Corynebacterium diphtheriae*, a Gram-positive, nonsporulating, uncapsulated, nonmotile bacillus, is the cause of diphtheria. In addition to causing cutaneous and invasive illnesses, it typically involves the lungs (respiratory diphtheria) and may be carried asymptomatically (Okamoto et al., 2018).

Waves of epidemic occurrence have historically been a feature of the ancient disease diphtheria, which has an increased prevalence and mortality rate (Zakikhany & Efstratiou, 2012). Before immunization, diphtheria was a common pediatric illness with a high fatality rate. In wealthy countries that have adopted toxoid immunization, diphtheria has become relatively rare, despite the fact that it is still very frequent in developing countries in Asia's southern and eastern regions (Okamoto et al., 2018). From nearly a million cases per year in the mid-1900s to 7097 incidents in 2016, the global epidemic of diphtheria, which was previously the most common cause of child mortality, has significantly decreased (Truelove et al., 2020).

Recently, advances in molecular science and structural biochemistry have led to the development of innovative pathways through which these diphtheria toxins induce cytotoxic effects. These innovations provide valuable opportunities for better targeted drug delivery and create new therapeutic agents against tumors and other diseases (Varol et al., 2012). Moreover, understanding the interaction with molecules and various cellular pathways opens new goals for toxin modification with enhanced selectivity for proper drug design and delivery (Huggins et al.,

2012). This study comprehensively explores the molecular mechanism of diphtheria toxin activity. It also explores its entry through receptors, intracellular pathways, and enzymatic interaction .

Types of Diphtheria

Asymptomatic Diphtheria

Although our patient reported a positive sputum analysis, the unintentional observation of a positive pharynx swab is typically indicative of asymptomatic carriage. There aren't many reports of this ailment (Scheifer et al., 2019). 22 out of 46 probable cases of diphtheria were verified by laboratory testing: 17 patients, comprising 4 who passed away, tested positive for both culture and PCR, while 5 patients, involving 1 who passed away, tested positive only for PCR. Ten patients for whom PCR findings were unavailable, seven suspected cases, and three in which the patient passed away were classified as epidemiologic. Two out of 49 benign contacts were verified as diphtheria carriers (Kitamura et al., 2020).

Respiratory Diphtheria

Toxin-producing *C. diphtheria* strains are the source of the potentially fatal bacterial illness known as respiratory diphtheria (Otshudiema et al., 2021). Prodromal symptoms of respiratory infections usually give way to tonsillar, laryngeal, or pharyngeal membrane inflammation (Truelove et al., 2020).

It is marked by a dense, dirty-grayish or darkish pseudomembrane that makes breathing difficult, as well as a foul odor (Weerasekera et al., 2019). Coughing can cause the pseudo-membrane to separate, which can lead to hemorrhage of the epithelial tissue. Decomposing erythrocytes can then cause the pseudo-membrane to become dirty and brownish. Dyspnea may progress to asphyxia and death if the inflammation spreads to the nasal passageways and larynx, obstructing the airways (Ott et al., 2022). Respiratory diphtheria is a nationally notifiable disease in the United States. The Institute for the Control of Diseases (CDC) receives samples from suspected cases for confirmation of species and toxin, and the CDC provides diphtheria antitoxin (DAT) for treatment (Otshudiema et al., 2021). A rare respiratory disease that mimics diphtheria is caused by *C. ulcerans* (Barroso et al., 2018).

Cutaneous Diphtheria

Diphtheria is more prevalent in tropical areas and can also manifest as cutaneous lesions, which are characterized by rolled-edge ulcers on exposed limbs, especially the legs (Gower et al., 2020). Interaction with lesions or contaminated secretions is the method of transmission for cutaneous diphtheria, which may be more contagious than respiratory diphtheria, according to some data (Koopman & Campbell, 1975). Patients may simultaneously have respiratory and cutaneous conditions (Gower et al., 2020). Injuries or bites from bugs are the usual entrance routes for cutaneous diphtheria, but other fluids and contaminated items may also serve as sources of infection (Zasada, 2015).

Molecular Pathways of Diphtheria Toxin

Structure of Diphtheria Toxin

Exotoxins are pathogen-derived proteins that generate the illness symptoms that come with many bacterial infections (Land, 2023). Learning the structures and activities of these remarkably deadly proteins has advanced significantly in the science of toxicology since the first bacterial exotoxin, diphtheria toxin (DT), was identified in 1888 as the cause of the main symptoms of diphtheria (Gopinathan & Maheswary, 2025). Despite having no obvious sequence similarities, the most powerful toxins, such as *Botulinum* toxin, tetanus toxin, and diphtheria toxin, all had three functional units in common: a receptor binding domain (R), a translocation domain (T), and a cytotoxic enzymatic domain (C) (Sugiman-Marangos et al., 2022). Understanding how this versatile protein attaches, penetrates, and induces host cells to finally cause disease has been revealed by almost one century of studying the form, activity, and pathways that govern the actions of DT (Sugiman-Marangos et al., 2022).

Production and Activation of Toxin

The severity of *C. diphtheriae* is linked to the metal ion-activated transcriptional regulator DtxR (Parveen et al., 2019). Each repressor monomer has two metal ion binding sites, according to structure determination. According to site-directed mutagenesis, target DNA repressor recognition depends on binding site 2 (primary) (Love & Murphy, 2006). Diphtheria is one of the most extensively researched bacterial infectious illnesses. The molecular reactions of each of these toxic compounds with eukaryotic cellular factors that are crucial for the quick shifting of their active sites by way of a tiny hole in the trans-endosomal vesicle membrane and their subsequent entry into the cell cytochrome P450 system have been the subject of extensive research in recent years (Murphy, 2011). The benchmark for the research of comparable bacterial protein toxins has been established by these seminal investigations of the antigen of toxigenic origin, *C. diphtheriae*, and its primary virulence determinant, diphtheria toxin (Yellaboina et al., 2004).

Mechanism of Action and Internalization

By attaching its segment B to the heparin-binding epidermal growth factor-like precursor (HB-EGF), diphtheria toxin is endocytosed into the cells (Mitamura et al., 1995). The domain C is broken down and moved into the cytosol in the late endosome's acidic environment. Once in the cytoplasm, the C domain stimulates the transfer of NAD's ADP-ribose moiety onto EF-2, making EF-2 inactive and, as a result, stopping the host cell's production of proteins (Pitard & Malliavin, 2019).

Systemic and Cellular Impacts

Respiratory failure, myocarditis, polyneuropathy, and renal damage brought on by the systemic spread of bacterial toxins are

usually linked to death. In addition to infecting skin wounds, *C. diphtheriae* and its related *C. ulcerans* induce cutaneous diphtheria, which is typified by deep ulcers and widespread cutaneous and dermal necrosis (Robinson et al., 2023). The mechanism of entry and its pathogenesis are shown in Figure 1.

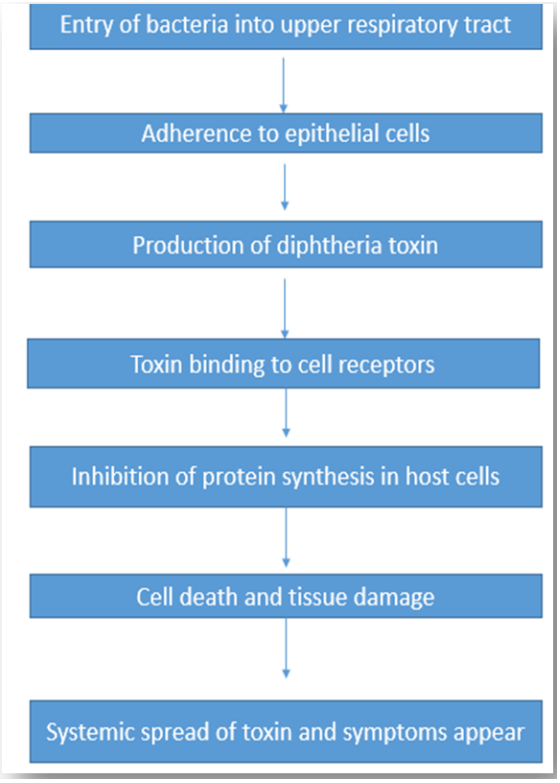

Fig. 1: Pathogenesis of diphtheria toxin: The bacteria enter through the nasal pathways and adhere to the epithelial cells. It then produces its toxins, which bind to the receptors on the cells and cause inhibition of protein synthesis, which causes cell death and tissue damage, resulting in the overall spread of the disease.

1. Pathophysiology of Diphtheria

Local Effects of Toxin

A strong diphtheria exotoxin's local and systemic effects, along with localized inflammation of the skin or respiratory tract, cause a symptomatic diphtherial infection (Gupta & Jayashree, 2017). The occurrence of a lysogenic bacteriophage carrying the toxin-encoding gene (tox+) is necessary for the generation of exotoxins (Casas & Maloy, 2011). There are three domains in all for the toxin. The catalytic unit, or fragment A, contains one domain, while fragment B contains the other two (one for interacting with receptors and one that might be involved in membrane insertion and translocation). When diphtheria toxin enters cells, it deactivates elongation factor 2, which results in cell death (Jørgensen et al., 2006). The path of cell entry is provided by the toxin's binding to a membrane receptor. Diphtheria toxin uses a growth factor progenitor as a receptor; the receptor for diphtheria toxin seems to be the same as the precursor of a growth factor that binds heparin (Barroso et al., 2018). The mechanism is shown in Figure 2

Systemic Complications

Since diphtheria rarely occurs in some regions of the nation with low vaccination rates, modern doctors have all but forgotten about the disease and its neurological effects (Mattos-Guaraldi et al., 2003). It is acknowledged that one of the most serious side effects of diphtheria is neurological impairment (Sanghi, 2014). Neurological consequences are multiphasic in development and correlate with the severity of the initial infection. The neurological symptoms are caused by *C. diphtheriae* exotoxins. There is little research on diphtheria's neurological effects. Because of the size of the membrane and the quantity of toxin ingested by Schwann cells, the pathology of diphtheria is dependent on the intensity of symptoms. It causes neurological symptoms by preventing myelin production (Prasad & Rai, 2018).

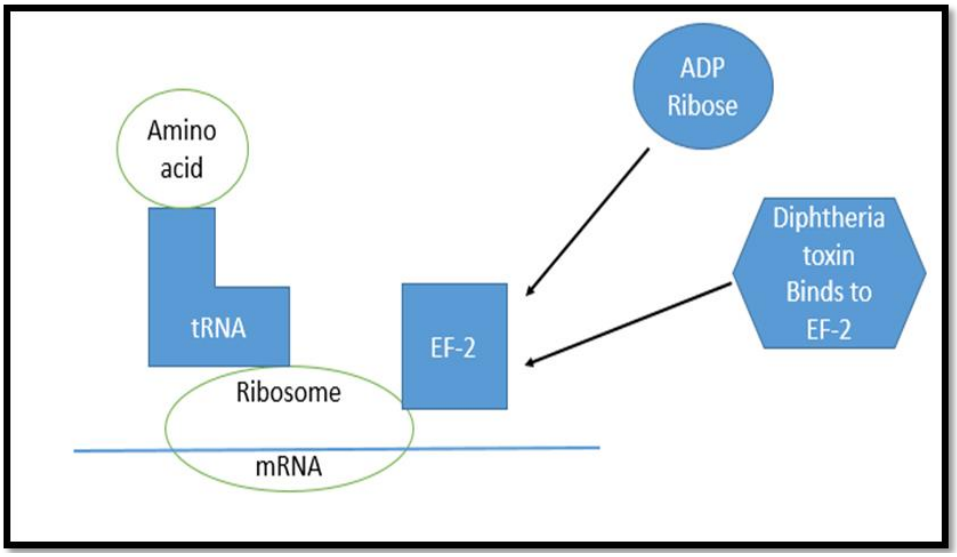

Fig. 2: ADP-ribosylation of elongation factor-2: The diphtheria toxin binds to the EF-2, which is necessary for translation, and causes its ADP-based ribosylation and blocks the translational process, resulting in cell death.

Severe cardiac contractility degradation is a hallmark of diphtheria's cardiac involvement, which can be reversed with effective treatment. About 10–25% of individuals with respiratory diphtheria may develop myocarditis, which has been linked to a high death rate (Samdani et al., 2018). ECG abnormalities indicate asymptomatic cardiac involvement, while heart failure symptoms indicate symptomatic involvement (Samdani et al., 2018).

Present-Day Therapeutic Strategies

Antitoxin Drug Therapy

The serum of inoculated animals is protected against DT, according to research done in 1890 by Shibasaburō Kitasato and Emil von Behring (Wenzel et al., 2020). This serum therapy, which won the first Nobel Award in medicine in 1901, was a breakthrough in the prevention and cure of diphtheria, particularly in children. Even with the implementation of successful vaccination campaigns, there are still gaps in immunization coverage, which means that in certain places, diphtheria is still endemic. Isolated occurrences still happen, even in communities with high vaccination rates (Wenzel et al., 2020).

Serum sickness is the term for the condition where the human immune system develops immune system antibodies contrary to foreign antigens introduced by administering animal sera (Casadevall & Scharff, 1994). This results in an accumulation of immune complexes that can develop in the joints or small vessels, thereby enabling the pathway of complements and starting an extensive and potentially dangerous inflammatory response. Due to manufacturing being stopped in a number of countries, DAT is currently in short supply and frequently inaccessible to patients (Casadevall & Scharff, 1994). New therapy strategies using recombinant, completely human antibodies are appealing because there exists a dire demand for another option to the equine DAT. Since they are proteins produced by humans, synthesized human antibodies are manufactured in cell culture, have a predetermined sequence, and do not cause serum sickness. Recombinant antibodies are perfect treatments against toxins and infections because of these benefits (Laustsen, 2019).

Antibiotic Therapy

In order to eradicate the toxic *Corynebacterium diphtheriae*, prevent the formation and transmission of the toxin in patients who exhibit symptoms and clinical illness, and lessen its propagation from silent carriers and its colonization of close contacts, antibiotics are required (Bonnet & Begg, 1999). According to the World Health Organization's (WHO) and the Centers for Disease Control and Prevention's (CDC) recommendations for the treatment of diphtheria, the empirical antibiotic groups penicillin and macrolide are used to eradicate toxic *C. diphtheria* (Osarenren et al., 2024). Since the 1940s, penicillin has been the accepted treatment for diphtheria. The rise in resistance isolates of this toxic bacterium has already been shown in a few investigations (Meera & Rajarao, 2014).

Although erythromycin has long been the standard treatment for diphtheria, a number of studies have shown that the drug's effectiveness has decreased. The gastrointestinal side effect of this medication is another issue. In the 1980s, there were reports of erythromycin's cardiac side effects. Nevertheless, no such information about diphtheria carriers and patients has been gathered and published in Indonesia. Only oral versions of erythromycin are available in the nation. Only a small number of clinicians currently utilize clarithromycin, which is also included in a few guidelines. This medication has a number of benefits. It is less expensive in Indonesia than azithromycin and has fewer gastrointestinal side effects than erythromycin (Husada et al., 2019).

Azithromycin has also been included by the WHO to the list of common medicines for diphtheria. Typhoid fever and other infectious disorders can be effectively treated with azithromycin. Although it is widely accessible and well-known by many practitioners, azithromycin is not currently utilized extensively for diphtheria sufferers in Indonesia (Polonsky et al., 2021).

Strategies for Vaccination

A biological preparation that stimulates the body's immune system to produce a specific to an antigen response to an antigen in order to prevent the ailment it causes is known as a vaccination. Usually, attenuated or inactivated forms of the pathogen or their derived proteins and polysaccharides are used to make vaccines (Borkar & Goenka, 2019). To guarantee that it produces an immune system reaction that is equally safe and protective, each vaccine is painstakingly created and put through a rigorous testing process (Montero et al., 2024). This emphasizes the complex interplay and balance between the immune system's dynamics and the vaccine's composition (Montero et al., 2024). Vaccines are regarded as landmarks in the history of medicine and public health and have been essential in the past 200 years to avoid the possibility of infectious diseases. According to WHO estimates, vaccinations presently avert between 3.5 and 5 million fatalities each year from illnesses like measles, influenza, tetanus, pertussis, and diphtheria (Montero et al., 2024).

Since 2005, the Advisory Committee on Immunization Practices (ACIP) has advised adults and adolescents to receive a single dose of the form of decreased diphtheria toxoid and an acellular pertussis (Tdap) vaccine (Havers, 2020). Booster doses of the Tetanus infection and diphtheria toxic substances (Td) vaccine are advised every ten years after receiving Tdap, or as needed for wound care. Previously, ACIP solely suggested Td; however, at its October 2019 meeting, the organization revised its guidelines to permit the use of Td or Tdap in two ways (Havers, 2020).

Most patients already have defenses against the toxin, as the entire population is typically inoculated against diphtheria. The immunotoxin may be neutralized by these antibodies, which would reduce the effectiveness of treatment. Multiple-course therapy regimens may be hampered by the development of defenses towards the toxic moiety during the course of treatment, even if the patient has no underlying immunity (Shafiee et al., 2019).

In the United Kingdom, the effectiveness of the initial dose of the diphtheria vaccination, which is assessed at one and two years of age, has currently been around 91% to 95% since early 1990 (Wagner, 2015). Preschool booster coverage was first assessed in 1999–2000; throughout the next ten years, it stayed between 78% and 82%, then rose to 86% in 2009–2010, and has since hovered between 84% and 89%. Adolescent booster coverage was first evaluated in 2016 and stayed within 83% to 88% until 2019 (Vusirikala et al., 2023). However, the COVID-19 pandemic has had a recent impact, causing this decline in 2020–2021 (Vusirikala et al., 2023). Strategies for diphtheria are shown in Figure 3.

Consequences for Drug Research

Targeting the Mechanisms of Toxins

The crystalline structure of eEF2 showed that it has five domains (Parikh & Schramm, 2004). Motifs IV and V constitute an extension, whereas the three N-terminal domains are comparable to eEF1A save for their distinctive mutation in the binding GTP domain. The general

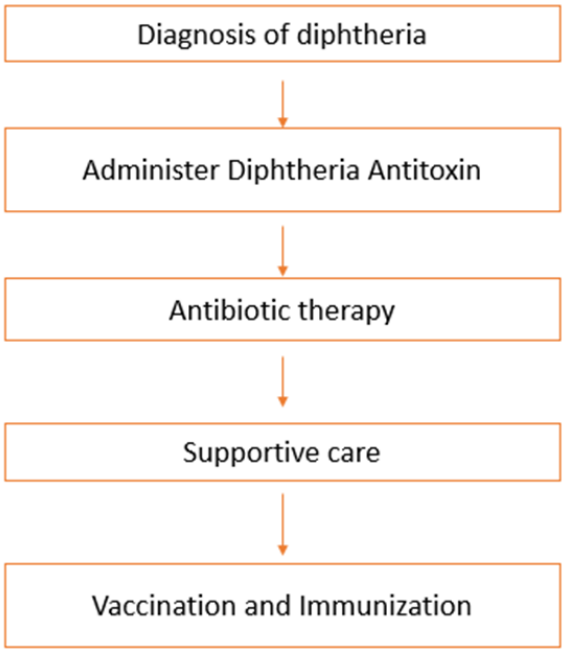

Fig. 3: Strategies for diphtheria patients: As soon as the disease is diagnosed, the antitoxin therapy is given, progressing towards antibiotic medication, and supportive care is provided with further vaccination.

structure of eEF2 is interesting because it resembles the eEF1A-aa-tRNA complex molecularly (Mateyak & Kinzy, 2013). Numerous bacterial infections that affect humans target translation elongation. The toxin produced by *Vibrio cholerae*, *Pseudomonas aeruginosa*, and *C. diphtheria* ADP-ribosylates (ADPR) eEF2 on the diphthamide residues in domain IV, hence inhibiting eEF2 action. For eEF2 to be ADPR by bacterial toxins, the diphthamide alteration must be present (Mateyak & Kinzy, 2013).

Toxin Inhibitors Development

The production of AB-type protein toxins, which effectively infiltrate cells of humans or animals and function as enzymes in their cytoplasm, is how many bacteria cause disease (Popoff, 2024). As a result, the specific toxin's clinical symptoms and disrupted cell functions occur (Popoff, 2024). As a result, compounds that especially target and counteract these poisons offer exciting new therapeutic possibilities. Here, we discovered that the FDA-approved medication disulfiram (DSF), which has been used for centuries to alleviate drinking disorders, protects cells from impairment with deadly toxin (LT) of *Bacillus anthracis*, known to cause anthrax, C2 enterotoxin in *Clostridium botulinum*, and the toxin that causes diphtheria (DT) of *Corynebacterium diphtheria*, the pathogenic agent of diphtheria, whenever used for the levels lesser than those that are detected in the plasma, of patients receiving average DSF medication to treat alcoholism (up to 20 μM) (Borho et al., 2024). Furthermore, copper, a known activator of DSF activity, increases this inhibitory impact (Borho et al., 2024).

Peptides with antimicrobial properties engage with the negatively charged coating of bacteria and create pores in microbial membranes because they are hydrophobic and cationic. There have also been reports of antimicrobial peptides' immunomodulatory effects and inhibition of cell wall production. Apart from their impact on bacteria, antimicrobial peptides—specifically, those belonging to the defensin family—have also been found to block bacterial toxins such as *Clostridium difficile* toxins and diphtheria toxin (Kordus et al., 2022).

Innovative Antitoxin Techniques

While anti-toxins tend to be proteins or short RNAs that neutralise the toxin or prevent its creation, toxins are always proteins that target particular intracellular targets (Duracova et al., 2018). Prokaryote genomes contain several genes that encode for toxin-antitoxin (TA) complexes, and many species have tens of chromosomal and plasmid TA loci. Based on their mechanism of action and genetic organization, the complexes are divided into three categories (Hayes & Van Melderen, 2011). Significant variation in TA combinations has resulted from the extensive evolutionary rearranging of toxin and antitoxin genes amongst the three categories of complex (Hayes & Van Melderen, 2011).

Rapid delivery of the diphtheria antitoxin (DAT) in conjunction with concurrent antibiotic therapy is the most efficient treatment for diphtheria (Both et al., 2014). One of the World Health Organization's Essential Medicines is DAT, a preparation of equine immunoglobulin. Essential medications should always be accessible in operational health systems in sufficient quantities, in suitable dosage forms, with guaranteed quality, and at costs that members of the public and individuals can afford (Both et al., 2014). However, due to low commercial viability, strict regulations for the safe production of blood-derived products, and halted production in numerous countries, DAT is in short supply and usually unavailable to patients. Although DAT is a fundamental component of diphtheria diagnosis, obtaining DAT for toxigenicity testing is a frequent challenge for a number of diagnostic reference laboratories in the EU and beyond. In general, it appears that DAT is not widely available for diagnosis as well as therapy. Therefore, in addition to attempts to enhance the present DAT supply, there is a need for expedited studies and the development of alternatives, such as molecular-based diagnostic techniques and monoclonal antibodies for therapy. Owing to the disease's rarity, it would be beneficial to keep track of DAT availability both within and between nations and to systematically manage a small stockpile for all EU nations (Both et al., 2014).

Early delivery of diphtheria antitoxin (DAT), which is made from immunized animals, is the most effective way to treat the disease. However, problems like the possibility of an allergic reaction, serum sickness, and anaphylaxis continue to restrict the usage of polyclonal antibody therapies to the management of diphtheria. Furthermore, immunoassays are frequently used to evaluate the effectiveness of different vaccinations and have occasionally been created expressly to replace in vivo effectiveness testing (Khalili et al., 2024).

Prospects for Vaccine Development

Traditionally, the primary target or targets of the effective immune response are determined by studying "protective" antibodies elicited by natural infection. These are typically proteins (toxins, like tetanus and diphtheria), or branched hemagglutinin in pertussis vaccines, or carbohydrates (capsular polysaccharides, like pneumococcus and meningococcus). The suitability of possible candidates as vaccine antigens is examined, including any inherent toxicity and their similarity with human proteins. Before an antigen (like pertussis toxin) can be safely given to people, detoxification may be necessary; however, some detoxification techniques may damage epitopes and affect immunogenicity (Cunningham et al., 2016).

Other vehicles that enable the close interaction of antigens, as well as immune modulators, have been developed concurrently with the advancement of improved adjuvants for vaccine delivery. Toxins, viruses, liposomes, immunostimulating complexes (ISCOMS), and micro- or nanoparticles are a few examples of these. While the remaining vaccinations have shown encouraging clinical evidence, just the initial two are currently included in commercially accessible vaccines (Rappuoli & Aderem, 2011).

Although they use different technologies than the ones mentioned above, toxoid-conjugated vaccines work on the same general principle: a detoxified but immunogenic microbial PAMP that can trigger innate and T-cell-dependent responses is combined with antigenic polysaccharide targets that can produce potent responses from B cells and antibody production. Using toxoids derived either from the toxin associated with diphtheria of *Corynebacterium diphtheria* or the tetanospasmin of *Clostridium tetani*, this method has been used to create licensed vaccines contrary to serious invasive bacterial infections. It has been demonstrated that these toxoids produce more robust and long-lasting responses to antibodies than the polysaccharide desirers alone, most likely as a result of the activation of T-cell assistance (Cunningham et al., 2016).

Current Research

Inhibitors of Small Molecules

Nicotinamide adenine dinucleotide (NAD+) is used as a co-substrate by diphtheria toxin-like ADP-ribosyl transferases (ARTDs), often referred to as poly (ADP-ribose) polymerases (PARPs), to transfer ADP-ribose to their target proteins (Poltronieri & Čerekovic, 2018). PARPs stimulate the creation of dynamic structures known as linear or branching poly (ADP-ribose) chains, which are identified by various reader domains and degraded by poly (ADP-ribose) glycohydrolases. Mono-ADP-ribosylation is catalyzed by a sizable portion of the PARP family, however, chain elongation is not. Functional data for these mono-ADP-ribosyltransferases (mARTDs) have been gathering lately. PARP inhibitors have been the focus of numerous drug discovery initiatives (Ekblad et al., 2015).

Advancements in Biotechnology

In addition to novel services for producing improved conventional vaccines, DNA vaccines, transcutaneous (microneedle-mediated) vaccines, oral vaccines, and edible vaccines expressed in transgenic plants, creativity includes biological inactivation of diphtheria toxoid, novel vaccine delivery mechanisms, brand-new additives in order (which include those TLR-independent and TLR-dependent adjuncts), and heat- and freeze-stable agents (Zafar et al., 2025). These developments aim to enhance vaccination quality (consistency, stability, safety, and efficacy), usability, and/or heat stability. Global coverage of the diphtheria vaccine should rise as a result of its effective development and application (Bae, 2011).

Particular Challenges and Future Paths

The Resistance Mechanisms

The medical establishment believed that the fight against infectious diseases was over with the advent of antibiotics (Reygaert, 2018). But now that a large number of bacteria have developed resistance to several antimicrobial treatments, the battle appears to have shifted in the bacterium's favor. Globally, infectious illnesses are presently the leading causes for morbidity and mortality (Reygaert, 2018). While erythromycin resistance has been identified, there is no description of β-lactam resistance in toxic diphtheria (Forde et al., 2021).

Patients now have fewer alternatives for therapy due to ongoing increases in antibiotic resistance, which has also increased morbidity and death (Cars & Nordberg, 2005). As a result, we are now dealing with more serious infections that require more involved care, as well as lengthier sickness courses that frequently call for prolonged hospital stays (Cars & Nordberg, 2005). The medical expenses linked to these illnesses have skyrocketed as a result. According to a conservative estimate from the CDC, antimicrobial-resistant diseases cause more than 2 million illnesses and more than 23,000 deaths annually in the United States (Solomon & Oliver, 2014).

The four primary categories of antimicrobial resistance mechanisms are: (1) restricting medication uptake; (2) altering a drug target; (3) rendering a drug inactive; and (4) active drug efflux. Limited intake, drug inactivity, and drug efflux are examples of intrinsic resistance mechanisms; drug target alteration, drug inactivity, and drug efflux are examples of acquired resistance mechanisms. Gram-negative and gram-positive bacteria employ different kinds of processes due to structural variations, among other factors. Gram-positive bacteria lack the ability to use some drug efflux mechanisms and are less likely to limit drug uptake due to their lack of an outer membrane made of LPS, while gram-negative bacteria use all four major mechanisms (Mahon & Lehman, 2022).

Safety and Effectiveness in Drug Design

In recent years, there have been high-profile drug withdrawals, higher development costs, and fewer new drug approvals despite advances in the genetic makeup of chemistry and protein engineering (Caskey, 2007). There is little information available on the safety and immunogenicity of the suggested revaccination regimen for adult recipients of transplantation of allogeneic stem cells (allo-HSCT) against the diseases polio, pertussis, tetanus, diphtheria, and hepatitis B (Conrad et al., 2020).

Conclusion

Although widespread immunization has significantly reduced its prevalence, diphtheria, a bacterial disease produced by *Corynebacterium diphtheriae*, has historically been a major cause of childhood mortality. It can still result in serious sickness, though, and is still prevalent in some poor areas. The Tdap vaccination is essential, and immunization continues to be the most successful preventative measure. With novel approaches like toxoid-conjugated vaccines, enhanced adjuvants, and cutting-edge delivery systems including genetic material, oral, and edible vaccines, vaccine development is still ongoing. The stability, effectiveness, and accessibility of vaccines are intended to be improved by these developments, particularly in regions with low resources. Antimicrobial resistance (AMR) is a growing issue in the fight against infectious

illnesses, including diphtheria. Multiple drug-resistant bacteria are making treatment options more difficult and increasing the risk of more serious infections, longer hospital stays, and higher healthcare expenses. Gram-negative bacteria tend to use more methods than gram-positive bacteria, although resistance mechanisms differ depending on the type of bacteria. of order to meet the changing difficulties of infectious diseases, the rise of AMR emphasizes the necessity of both new therapeutic approaches and ongoing advancements in vaccine research.

References

Bae, K. D. (2011). The evolution and value of diphtheria vaccine. *KSBB Journal*, *26*(6), 491-504.

Barroso, L. F., Pegram, P. S., & Kaplan, S. (2018). Epidemiology and pathophysiology of diphtheria. *UpToDate*.

Bonnet, J., & Begg, N. (1999). Control of diphtheria: guidance for consultants in communicable disease control. *Communicable disease and public health*, *2*, 242-249.

Borho, J., Kögel, M., Eckert, A., & Barth, H. (2024). Repurposing FDA-approved disulfiram for targeted inhibition of diphtheria toxin and the binary protein toxins of Clostridium botulinum and Bacillus anthracis. *Frontiers in Pharmacology*, *15*, 1455696.

Borkar, T. G., & Goenka, V. (2019). Techniques employed in production of traditional vaccines commonly used by military forces: A review. *Journal of Archives in Military Medicine*, *7*(1-2).

Both, L., White, J., Mandal, S., & Efstratiou, A. (2014). Access to diphtheria antitoxin for therapy and diagnostics. *Eurosurveillance*, *19*(24), 20830.

Cars, O., & Nordberg, P. (2005). Antibiotic resistance–The faceless threat. *International Journal of Risk & Safety in Medicine*, *17*(3-4), 103-110.

Casadevall, A., & Scharff, M. D. (1994). Serum therapy revisited: animal models of infection and development of passive antibody therapy. *Antimicrobial agents and chemotherapy*, *38*(8), 1695-1702.

Casas, V., & Maloy, S. (2011). Role of bacteriophage-encoded exotoxins in the evolution of bacterial pathogens. *Future microbiology*, *6*(12), 1461-1473.

Caskey, C. T. (2007). The drug development crisis: efficiency and safety. *Annu. Rev. Med.*, *58*(1), 1-16.

Chaudhary, A., & Pandey, S. (2020). Corynebacterium diphtheriae.

Conrad, A., Perry, M., Langlois, M.-E., Labussière-Wallet, H., Barraco, F., Ducastelle-Leprêtre, S.,...Chidiac, C. (2020). Efficacy and safety of revaccination against tetanus, diphtheria, haemophilus influenzae type b and hepatitis B virus in a prospective cohort of adult recipients of allogeneic hematopoietic stem cell transplantation. *Biology of Blood and Marrow Transplantation*, *26*(9), 1729-1737.

Cunningham, A. L., Garçon, N., Leo, O., Friedland, L. R., Strugnell, R., Laupèze, B.,...Stern, P. (2016). Vaccine development: From concept to early clinical testing. *Vaccine*, *34*(52), 6655-6664.

Duracova, M., Klimentova, J., Fucikova, A., & Dresler, J. (2018). Proteomic methods of detection and quantification of protein toxins. *Toxins*, *10*(3), 99.

Ekblad, T., Lindgren, A. E., Andersson, C. D., Caraballo, R., Thorsell, A.-G., Karlberg, T.,...Elofsson, M. (2015). Towards small molecule inhibitors of mono-ADP-ribosyltransferases. *European journal of medicinal chemistry*, *95*, 546-551.

Forde, B. M., Henderson, A., Playford, E. G., Looke, D., Henderson, B. C., Watson, C.,...Muttaiyah, S. (2021). Fatal respiratory diphtheria caused by ß-lactam–resistant Corynebacterium diphtheriae. *Clinical Infectious Diseases*, *73*(11), e4531-e4538.

GOPINATHAN, A., & MAHESWARY, D. (2025). CORYNEBACTERIUM DIPHTHERIAE PITTALA KIRANMAI. *Bacterial Enemies of Human Health*, 293.

Gower, C. M., Scobie, A., Fry, N. K., Litt, D. J., Cameron, J. C., Chand, M. A & Ramsay, M. E. (2020). The changing epidemiology of diphtheria in the United Kingdom, 2009 to 2017. *Eurosurveillance*, *25*(11), 1900462.

Gupta, S., & Jayashree, M. (2017). Diphtheria: Relic or relevant. *Journal of Pediatric Critical Care*, *4*(3), 85-90.

Havers, F. P. (2020). Use of tetanus toxoid, reduced diphtheria toxoid, and acellular pertussis vaccines: updated recommendations of the Advisory Committee on Immunization Practices—United States, 2019. *MMWR. Morbidity and mortality weekly report*, *69*.

Hayes, F., & Van Melderen, L. (2011). Toxins-antitoxins: diversity, evolution and function. *Critical reviews in biochemistry and molecular biology*, *46*(5), 386-408.

Huggins, D. J., Sherman, W., & Tidor, B. (2012). Rational approaches to improving selectivity in drug design. *Journal of medicinal chemistry*, *55*(4), 1424-1444.

Husada, D., Soegianto, S. D. P., Kurniawati, I. S., Hendrata, A. P., Irawan, E., Kartina, L.,...Ismoedijanto. (2019). First-line antibiotic susceptibility pattern of toxigenic Corynebacterium diphtheriae in Indonesia. *BMC infectious diseases*, *19*, 1-11.

Jørgensen, R., Merrill, A. R., & Andersen, G. R. (2006). The life and death of translation elongation factor 2. *Biochemical Society Transactions*, *34*(1), 1-6.

Khalili, E., Lakzaei, M., & Aminian, M. (2024). Neutralizing anti-diphtheria toxin scFv produced by phage display. *Biotechnology Letters*, *46*(3), 385-398.

Kitamura, N., Le, T. T., Le, L. T., Nguyen, L. D., Dao, A. T., Hoang, T. T.,...Do, H. M. (2020). Diphtheria outbreaks in schools in central highland districts, Vietnam, 2015–2018. *Emerging Infectious Diseases*, *26*(3), 596.

Koopman, J. S., & Campbell, J. (1975). The role of cutaneous diphtheria infections in a diphtheria epidemic. *Journal of infectious diseases*, *131*(3), 239-244.

Kordus, S. L., Thomas, A. K., & Lacy, D. B. (2022). Clostridioides difficile toxins: mechanisms of action and antitoxin therapeutics. *Nature Reviews Microbiology*, *20*(5), 285-298.

Land, W. G. (2023). *Damage-Associated Molecular Patterns in Human Diseases: Volume 3: Antigen-Related Disorders*. Springer.

Laustsen, A. H. (2019). How can monoclonal antibodies be harnessed against neglected tropical diseases and other infectious diseases? *Expert opinion on drug discovery*, *14*(11), 1103-1112.

Love, J. F., & Murphy, J. R. (2006). Corynebacterium diphtheriae: iron-mediated activation of DtxR and regulation of diphtheria toxin expression. *Gram-Positive Pathogens*, 726-737.

Mahon, C. R., & Lehman, D. C. (2022). *Textbook of Diagnostic Microbiology-E-Book: Textbook of Diagnostic Microbiology-E-Book*. Elsevier Health Sciences.

Mateyak, M. K., & Kinzy, T. G. (2013). ADP-ribosylation of translation elongation factor 2 by diphtheria toxin in yeast inhibits translation and cell separation. *Journal of Biological Chemistry*, *288*(34), 24647-24655.

Mattos-Guaraldi, A. L., Moreira, L. O., Damasco, P. V., & Hirata Júnior, R. (2003). Diphtheria remains a threat to health in the developing world: an overview. *Memórias do Instituto Oswaldo Cruz*, *98*, 987-993.

Meera, M., & Rajarao, M. (2014). Diphtheria in Andhra Pradesh–a clinical-epidemiological study. *International Journal of Infectious Diseases*, *19*, 74-78.

Mitamura, T., Higashiyama, S., Taniguchi, N., Klagsbrun, M., & Mekada, E. (1995). Diphtheria Toxin Binds to the Epidermal Growth Factor (EGF)-like Domain of Human Heparin-binding EGF-like Growth Factor/Diphtheria Toxin Receptor and Inhibits Specifically Its Mitogenic Activity (∗). *Journal of Biological Chemistry*, *270*(3), 1015-1019.

Montero, D. A., Vidal, R. M., Velasco, J., Carreño, L. J., Torres, J. P., Benachi O, M. A.,...O'Ryan, M. (2024). Two centuries of vaccination: historical and conceptual approach and future perspectives. *Frontiers in public health*, *11*, 1326154.

Murphy, J. R. (2011). Mechanism of diphtheria toxin catalytic domain delivery to the eukaryotic cell cytosol and the cellular factors that directly participate in the process. *Toxins*, *3*(3), 294-308.

Okamoto, K., Hatakeyama, S., Sugita, C., Ogura, K., Ueda, R., Kouda, H., & Nakata, J. (2018). Nasal diphtheria (chronic carriage) caused by nontoxigenic Corynebacterium diphtheriae. *Journal of infection and chemotherapy*, *24*(9), 759-762.

Osarenren, J., Omosigho, P. O., & Okesanya, O. J. (2024). Global strategies for addressing diphtheria resurgence epidemiology clinical impact and prevention. *Discover Public Health*, *21*(1), 1-11.

Otshudiema, J. O., Acosta, A. M., Cassiday, P. K., Hadler, S. C., Hariri, S., & Tiwari, T. S. (2021). Respiratory illness caused by Corynebacterium diphtheriae and C. ulcerans, and use of diphtheria antitoxin in the United States, 1996–2018. *Clinical Infectious Diseases*, *73*(9), e2799-e2806.

Ott, L., Möller, J., & Burkovski, A. (2022). Interactions between the re-emerging pathogen Corynebacterium diphtheriae and host cells. *International Journal of Molecular Sciences*, *23*(6), 3298.

Parikh, S. L., & Schramm, V. L. (2004). Transition state structure for ADP-ribosylation of eukaryotic elongation factor 2 catalyzed by diphtheria toxin. *Biochemistry*, *43*(5), 1204-1212.

Parveen, S., Bishai, W. R., & Murphy, J. R. (2019). Corynebacterium diphtheriae: diphtheria toxin, the tox operon, and its regulation by Fe2+ activation of apo-DtxR. *Microbiology spectrum*, *7*(4), 10.1128/microbiolspec. gpp1123-0063-2019.

Pitard, I., & Malliavin, T. E. (2019). Structural biology and molecular modeling to analyze the entry of bacterial toxins and virulence factors into host cells. *Toxins*, *11*(6), 369.

Polonsky, J. A., Ivey, M., Mazhar, M. K. A., Rahman, Z., le Polain de Waroux, O., Karo, B.,...Diaz, J. (2021). Epidemiological, clinical, and public health response characteristics of a large outbreak of diphtheria among the Rohingya population in Cox's Bazar, Bangladesh, 2017 to 2019: A retrospective study. *PLoS medicine*, *18*(4), e1003587.

Poltronieri, P., & Čerekovic, N. (2018). Roles of nicotinamide adenine dinucleotide (NAD+) in biological systems. *Challenges*, *9*(1), 3.

Popoff, M. R. (2024). Overview of bacterial protein toxins from pathogenic bacteria: mode of action and insights into evolution. *Toxins*, *16*(4), 182.

Prasad, P. L., & Rai, P. L. (2018). Prospective study of diphtheria for neurological complications. *Journal of pediatric neurosciences*, *13*(3), 313-316.

Rappuoli, R., & Aderem, A. (2011). A 2020 vision for vaccines against HIV, tuberculosis and malaria. *Nature*, *473*(7348), 463-469.

Reygaert, W. C. (2018). An overview of the antimicrobial resistance mechanisms of bacteria. *AIMS microbiology*, *4*(3), 482.

Robinson, K. S., Toh, G. A., Firdaus, M. J., Tham, K. C., Rozario, P., Lim, C. K.,...Mayer, J. (2023). Diphtheria toxin activates ribotoxic stress and NLRP1 inflammasome-driven pyroptosis. *Journal of Experimental Medicine*, *220*(10).

Samdani, S., Jain, A., Meena, V., & Meena, C. (2018). Cardiac complications in diphtheria and predictors of outcomes. *International Journal of Pediatric Otorhinolaryngology*, *104*, 76-78.

Sanghi, V. (2014). Neurologic manifestations of diphtheria and pertussis. *Handbook of clinical neurology*, *121*, 1355-1359.

Scheifer, C., Rolland-Debord, C., Badell, E., Reibel, F., Aubry, A., Pérignon, A.,...Caumes, E. (2019). Re-emergence of Corynebacterium diphtheriae. *Medecine et maladies infectieuses*, *49*(6), 463-466.

Shafiee, F., Aucoin, M. G., & Jahanian-Najafabadi, A. (2019). Targeted diphtheria toxin-based therapy: a review article. *Frontiers in microbiology*, *10*, 2340.

Solomon, S. L., & Oliver, K. B. (2014). Antibiotic resistance threats in the United States: stepping back from the brink. *American family physician*, *89*(12), 938-941.

Sugiman-Marangos, S. N., Gill, S. K., Mansfield, M. J., Orrell, K. E., Doxey, A. C., & Melnyk, R. A. (2022). Structures of distant diphtheria toxin homologs reveal functional determinants of an evolutionarily conserved toxin scaffold. *Communications Biology*, *5*(1), 375.

Truelove, S. A., Keegan, L. T., Moss, W. J., Chaisson, L. H., Macher, E., Azman, A. S., & Lessler, J. (2020). Clinical and epidemiological aspects of diphtheria: a systematic review and pooled analysis. *Clinical Infectious Diseases*, *71*(1), 89-97.

Varol, B., Bektaş, M., Nurten, R., & Bermek, E. (2012). The cytotoxic effect of diphtheria toxin on the actin cytoskeleton. *Cellular & molecular biology letters*, *17*, 49-61.

Vusirikala, A., Tonge, S., Bell, A., Linley, E., Borrow, R., O'Boyle, S.,...Amirthalingam, G. (2023). Reassurance of population immunity to

diphtheria in England: Results from a 2021 national serosurvey. *Vaccine*, *41*(46), 6878-6883.
Wagner, K. S. (2015). *Diphtheria epidemiology in the UK and Europe* Manchester Metropolitan University].
Weerasekera, D., Hahn, J., Herrmann, M., & Burkovski, A. (2019). Induction of necrosis in human macrophage cell lines by Corynebacterium diphtheriae and Corynebacterium ulcerans strains isolated from fatal cases of systemic infections. *International Journal of Molecular Sciences*, *20*(17), 4109.
Wenzel, E. V., Bosnak, M., Tierney, R., Schubert, M., Brown, J., Dübel, S.,...Hust, M. (2020). Human antibodies neutralizing diphtheria toxin in vitro and in vivo. *Scientific reports*, *10*(1), 571.
Yellaboina, S., Ranjan, S., Chakhaiyar, P., Hasnain, S. E., & Ranjan, A. (2004). Prediction of DtxR regulon: identification of binding sites and operons controlled by Diphtheria toxin repressor in Corynebacterium diphtheriae. *BMC microbiology*, *4*, 1-8.
Zafar, S., Akhtar, A., Sayed, E., Onaiwu, E., Arshad, M. S., & Ahmad, Z. (2025). Vaccine formulation design: challenges and opportunities. *RSC Pharmaceutics*.
Zakikhany, K., & Efstratiou, A. (2012). Diphtheria in Europe: current problems and new challenges. *Future microbiology*, *7*(5), 595-607.
Zasada, A. A. (2015). Corynebacterium diphtheriae infections currently and in the past. *Przegl Epidemiol*, *69*(3), 439-444