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**Dates:** Received: 01 September, 2017; Accepted: 28 October, 2017; Published: 30 October, 2017

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**Keywords:** *Corynebacterium diphtheriae*; Vaccination; Drug-resistant; Therapeutic targets

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## Research Article

# Pathogenesis of *Corynebacterium diphtheriae* and available vaccines: An Overview

## Abstract

*Corynebacterium diphtheriae* is Gram-positive bacteria responsible for causing diphtheria in human and once regarded for high mortalities worldwide. The fatality gradually decreased with improved living standards and further alleviated when many immunization programs were introduced. Public infectious diseases have positively been controlled by vaccination, yet, the importance of vaccination usually remained unnoticed for the adults. Many of the under-development vaccines are directed towards the childhood immunization. However, numerous drug-resistant strains emerged recently that consequently decreased the efficiency of current therapeutics and vaccines, thereby obliging the scientific community to start investigating new therapeutic targets in pathogenic microorganisms. In this study, we try to put together a short information regarding pathogenesis of *Corynebacterium diphtheriae* and reported vaccine till the date. Furthermore, we highlighted the emerging technique for identification of new therapeutic targets.

## Introduction

*Corynebacterium diphtheriae* is a human pathogen, responsible for causing Diphtheria. It was once an important cause of death worldwide. The mortality rates gradually decrease with time in the twentieth century in countries where living standards were improved, and then intensely fell once after the introduction of immunization programs [1]. Though, even today, despite these events it remains a substantial pathogen in many parts of the world. A variety of mechanisms are responsible for causing death. However, the name 'strangling angel' of children arose from the wing-shaped pseudo-membranes that form in the oropharynx. Displacement and impaction of these pseudo-membranes starts acute airway obstruction and sudden death [2,3]. Since there has been a revival of cases of non-lethal and lethal diphtheria in some countries in past few years and that considerable population displacements are happening due to refugee and immigration movements, more cases may be encountered in future.

Diphtheria is a potentially a lethal disease that mainly effect upper respiratory tract tissues and kills its sufferers slowly by suffocation. In 1884, a German physician, Edwin Klebs (1834–1913), was able to isolate the bacteria successfully that proved to be the etiological agent of the disease. It was later verified that toxin production begun only after the bacteria were infected by a specific virus or a bacteriophage themselves, carrying the toxin's genetic instructions [4].

Although diphtheria is forgotten disease in several European countries but it remains a potential health issue in many endemic countries and serious problem for those countries that are considered to be free from diphtheria [5]. In past few years, the awareness has been increased due to some periodic cases reported in Europe, especially contemporary case in Spain and cutaneous diphtheria cases in immigrants in Denmark, Germany and Sweden; the shortage issue of diphtheria antitoxin was also emphasized as a European Union priority [5]. Similarly, according to a report of WHO, cases were reported from some Asian countries. In 2015, India was found to have 2,365 cases of diphtheria. The number of reported cases were higher than any other country [1].

Public infectious diseases have positively been controlled by vaccination, yet, the importance of vaccination usually remained unnoticed for the adults [6]. Many of the under development vaccines are directed towards the childhood immunization [7]. The global population of elderly people has been increasing due to better-quality hygiene and healthcare system. Taking in account aging factor and the ongoing debility of the immune response to vaccination, it is important to develop worldwide strategies of vaccination and emphasize more on adult vaccination [1].

The increase in resistant strains of pathogens, we are in need of new tools to treat and avert the infectious diseases. The

extensive use of antibiotics in the medication of human and veterinary for many years has led to the development of bacterial strains that are multi-resistant with partial to no response to existing cures. It resulted in patients needing longer time for treatment as they need screening and treatment with several antibacterial agents and ultimately causing extra stress on patients and providers of health care [8]. The advancement of new technology and affectedly decreases in the running costs of NGS has allowed to generate a bulk amount of data on different pathogens [9,10]. NGS allows the analysis of microbial genome on broad-spectrum. This data has been proven to be valuable for infection prevention measures with the consequences for interspecies transmission, microbial evolution, variable regions and the potential of spread to different hosts [11-13]. The microbial pan-genome deals with the characterization of genomes by comparison of related species, possibly by accessing core, accessory and strain-specific genes [14].

The genetic knowledge of the *C. diphtheriae* species by performing comparative analysis of the complete genome sequences using pan-genomics technique by Trost et al., 2012 to describe the role of *C. diphtheriae* for its Genomic Diversity in the Cases of Classical Diphtheria, Endocarditis, and Pneumonia genomes at specie level. This data on the genomic content of different strains of *C. diphtheriae* provides deep insight into the virulence factors and features associated with the life style of the Human pathogen [15].

Here, we try to gather a short information regarding pathogenesis of *Corynebacterium diphtheriae* and reported vaccine till the date. Furthermore, we highlighted the emerging technique for identification of new therapeutic targets via integrated omics.

## Pathogenesis

Diphtheria toxin is responsible for the pathological consequences of diphtheria infection. The time of incubation may be from one to eight days, but mostly, it is between two to five days. The beginning is usually nonspecific with a sore throat and a low-grade fever which may mimic streptococcal pharyngitis, candidiasis or infectious mononucleosis [2,16,17]. It takes almost 24 hours, the gray colored pseudo-membrane appears covering the soft palate, uvula, and tonsils. It seems white initially but darkens as blood trickles into it. In young children, a more severe form occurs known as malignant or "bull neck" diphtheria. The commencement of diphtheria is quick and the growth of the pseudo-membrane faster associated with the buccal cavity, entire pharynx, middle ear and nose [2]. The soft palate, tonsils, and uvula may suffer necrosis and slough, and necrotic lesions may breach into the primary skeletal muscle with marked hemorrhage [18]. Furthermore, thinner pseudo-membranes are developed by distal airway, and the lungs are edematous and hemorrhagic [19]. The inner layer of Pseudo-membranes may have fibrin with an outer covering of neutrophils with aggregates of embedded bacteria inside the necrotic material [18]. There is clear inflammation of the cervical lymph nodes and adjacent soft tissues making the characteristic "bull neck" appearance. The firmness of the jugular veins may cause marked blocking of the face [2], other

portions of the upper airway may be involved with nasal and laryngeal diphtheria, and latter allied with a high death rate.

The heart could have pale, enlarged, chambers with a distinctive "streaky" appearance. Histologic sections may show marked hyaline degeneration that causes cell death with mononuclear cell permeation and lipid vacuoles inside surviving myocytes [18]. Proper treatment needs rapid management of diphtheria antitoxin and antibiotic coverage [17]. Shallow mucosal erosions may be present inside the stomach, and non-lethal diphtherial contagions of the skin can be found in the tropics. While these may lead to pharyngeal involvement through autoinfection [3]. Other sites of infection involve mycotic aneurysm formation, osteomyelitis, septic arthritis and splenic abscess [20].

Nowadays, we often think of cutaneous diphtheria in the context of umbilical diphtheria, wound diphtheria or impetiginous diphtheria. Owing to the ability of *C. diphtheriae* to colonize, lesions of skin can be different from any skin lesion of other origin (e.g., surgical wounds, eczema, pyoderma, impetigo, insect bites or dermatitis). Usually, an ulcerative lesion (ecthyma diphtheriticum) is the presenting lesion. It starts as a vesicle or abscess filled with straw-colored fluid, which breaks down soon after the formation. The lesion develop as punched-out ulcer, one or more than one, measuring from millimeters to a few centimeters, with margins slightly curved and raised. Furthermore, the margins may slightly be diluted or inverted. Lower legs, feet, and hands are the common sites for diphtheric lesions. The lesions are usually responsible for causing a lot of pain and may be covered with a dark pseudomembrane during the first couple of weeks. With the time, the lesion becomes sedative and the pseudomembrane falls away. The wound looks like a hemorrhagic base, usually with serous or serosanguinous exudate oozing from it. The tissues surrounding the infection stay edematous and pink, purple or livid in color and may show swellings or bullae. Skin lesions yielding *C. diphtheriae* on cultures are indistinguishable from those linked with other bacteria and can include nearly healed, dry or scaly lesions [21].

Diphtherial infections are mainly because of the toxin. Diphtheria toxin is an exotoxin secreted by *C. diphtheria*. Diphtheria toxin is a single polypeptide chain of 535 amino acids consisting of two subunits linked by disulfide bridges, known as an A-B toxin. There are at least four main steps involved in intoxication of a single eukaryotic cell by diphtheria toxin: (1) the binding of the toxin to surface receptor of its target cell; (2) grouping of charged receptors into layered pits and internalization of the toxin by receptor-mediated endocytosis; followed by acidification of the endocytic vesicle by a membrane-associated, ATP-driven proton pump, (3) the insertion of the transmembrane domain (B-subunit) into the membrane and smoothed the delivery of catalytic domain (A-subunit) to the cytosol, and (4) the ADP-ribosylation of elongation factor 2 (EF-2), which results in the permanent inhibition of protein synthesis as shown in figure 1. A single molecule of the catalytic domain delivered to the cytosol is enough to be deadly for the cell [22].

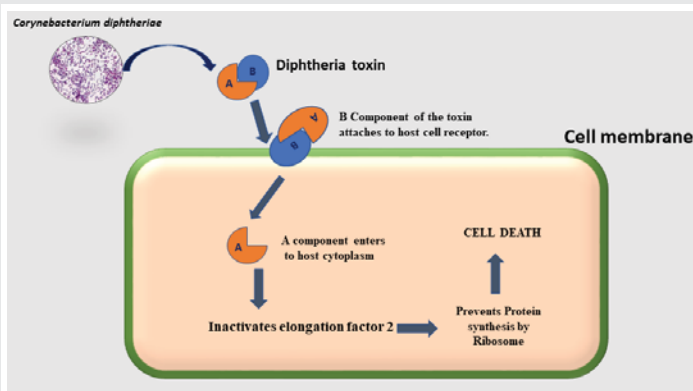


Figure 1: Mechanism of action of diphtheria toxin.

## Discovery of Diphtheria Toxin and Vaccine development

During the 19<sup>th</sup> century, diphtheria toxin was discovered by Emile Roux. This discovery led to the development of passive serum cures through the scientific contributions of many other scientists of that era <http://www.immunize.org/timeline/>. Likewise, the etiological agent of Pertussis, commonly known as the “a whooping cough,” was found to be a bacterium isolated from tissues of an infected patient in 1906 [23]. They revealed that the animal’s serum that had been exposed to sub-lethal doses of the bacteria involved in tetanus and diphtheria was defensive against the fatal effects allied with these pathogens by having an antitoxin outcome when injected into another animal. Furthermore, this discovery, which resulted in Behring inaugural Nobel Prize for Physiology and Medicine in 1901, was the idea of passive transmission in addition to serum therapy. He verified that serum could be attained from immune animals and transmitted to others as protection [24]. Once this concept made its way to clinical practice in late 19<sup>th</sup> century, handling problems were encountered while developing the right antitoxin concentration and potency. Consequently, in the early twentieth century, the U.S. Congress passed the Biologics Control Act legislation “to normalize the sale of viruses, serums, toxins, and similar products” to guarantee medication quality control.

However, with the growing use and fame of antitoxins derived from animal serum, scientists began to observe a syndrome, now known as serum sickness, or a reaction to immune-complexes designed from combining high concentrations of antigens with antibodies. This ultimately headed towards the use of human rather than animal serum, so it could reduce the rate of hostile events; still, treatment with serum was not perfect in controlling disease due to the frequency of hostile events and its short-lived period of action. Afterward, conjoining diphtheria toxin and antitoxin in the same syringe showed much more activity in reducing the mortality rate. The commercial availability of this combination became in 1897. This was the primary step towards passive to active immunization [4].

Later in the 20<sup>th</sup> century, a French veterinarian Gaston Ramon (1886–1963), working at the Pasteur Institute, used a diphtheria toxoid, produced by formalin and heat inactivation

without the use of antitoxin to securely tempt active immunity in humans [24]. This product was named anatoxin and provided the basis for the unique and clinically effective toxoid vaccine against diphtheria. Many experiments were performed to enhance the strength of the defensive response to the vaccine, and in 1926, the prominence of aluminum salts as an adjuvant added to the vaccine to augment the immune response to the antigen became apparent [1]. This discovery was made by Alexander Thomas Glenny (1882–1965) who proved that the toxoid only produced a lower level of antibody and immunity than desired, whereas improved immunity was accomplished when an inflammatory reaction was activated. With these substantial advances, tetanus and diphtheria toxoids became regularly used across America and Europe in the era between the 1930s and 1940s [4].

Since then, modifications have been made to these vaccines to produce higher purity and decrease the number of booster doses. Currently, extensive childhood vaccination is reducing the load of these diseases. Though this is a huge advantage, vaccines may produce antagonistic effects that can discourage their approval by some populations. This has directed to some safety movements which concluded in the congressionally legislated National Childhood Vaccine Injury Act in the 1980s it was shaped to recompense families for selected adverse events possibly related to compulsory childhood vaccinations [25]. Still, worldwide endorsements continue to call for routine immunization of children against diphtheria, tetanus, and pertussis with the combined DTP vaccine to ensure immunity in childhood and youth. Hence, DTaP has become one of the globally used vaccines to achieve extensive immunity across age groups [4].

Currently, in the United States, the pediatric formulation (diphtheria-tetanus-acellular pertussis [DTaP]) of vaccines are available for use under the brand names as Infanrix and Daptacel (Manufactured by GlaxoSmithKline and Sanofi Pasteur respectively). Teenage and adult formulation (tetanus-diphtheria-acellular pertussis [Tdap]) of vaccines which were approved in 2005 for teenagers are in use under the brand names as Boostrix (GlaxoSmithKline) and Adacel (Sanofi Pasteur) in the United States. Later in 2006, Tdap vaccination was recommended for adults younger than 65 years. These adult form of vaccines have an equal amount of tetanus and diphtheria toxoid in comparison with the adult form of Td vaccines. Boostrix has a reduced quantity of pertussis antigens compared with the Infanrix and it is licensed for persons 10 years of age and older. Adacel has a reduced quantity of pertussis toxin compared with Daptacel and is licensed for persons 10 through 64 years of age [26].

It has been reported in several studies that protective antibody levels of tetanus and diphtheria wilted since the last vaccination because of aging, and antibodies had on estimated the half-life of 11 years [6,27]. These data better explain the reason behind the lack of antibody in the older age. Due to work related pressure and military services, it had been observed in males from  $\geq 25$  years of age that antibody levels were significantly higher [28].

The immunization of DNA with in vivo electroporation is an alternative and competent approach to produce monoclonal antibodies (mAb). The mAb generation by DNA immunization is a novel approach to outwit the following technical hurdles associated with problematic antigens: low profusion and protein instability and use of recombinant proteins that lack posttranslational modifications [29].

Till the date, diphtheria has been very effectively controlled by an efficient immunization program in developed countries [30]. Though, in recent years, the disease has made a dramatic return, especially within Eastern Europe. The major outbreak since the beginning of mass immunization within Russia and the states that were newly independent of the former Soviet Union in the 1990s [31,32]. Furthermore, cases were reported from some part of Africa and Asia in the recent past [32,33].

In 2003, the genome (*C. diphtheriae* NCTC 13129) was sequenced at the Sanger Institute, which was clinical isolate related to this outbreak. It provided useful basis to identify candidate virulence factors besides the toxin itself, like iron transport systems and fimbrial proteins [34]. Jamal *et al.*, 2017 utilized the genomic information from all the available genomes and adopted an integrative OMICs approach for therapeutic target identification against diphtheria. In their work, high throughput comparative modeling (Pan-modelome) was performed to generate 3D structures from the proteome of all the available genomes. After filtering intra-specie conserved proteins, a final set of eight proteins (glpX, nusB, rpsH, hisE, smpB, bioB, DIP1084, and DIP0983) were identified as essential and non-host homologs, considering human as a host. The identified 8 proteins were subjected to virtual screening against four different compound libraries (extracted from the ZINC database, plant-derived natural compounds and Di-terpenoid Iso-steviol derivatives). The proposed ligand molecules showed lowered energy values, high complementarity with the predicted targets and favorable interactions. Interestingly, among the drug-like molecule from all the four databases, ZINC13142972 (1-[(2S, 3S, 4S, 5R)-3,4-dihydroxy-5(hydroxymethyl) oxolan-2-yl]imidazo[1,2-b]pyrazole-7-carbonitrile) showed good results against two of our predicted targets NP\_939302.1 (glpX, Fructose 1,6-bisphosphatase II) and NP\_939445.1 (DIP1084, Putative iron transport membrane protein, FecCD-family). Furthermore, Jacarandic Acid and Rhein were identified as the top ranked molecules from the library of natural compounds (28 molecules). The in-silico analysis of the library (derivatives of diterpenoid isosteviol) suggest that compounds 16-hydroxyisosteviol, 16-hydrazonisosteviol, 17-hydroxyisosteviol, 16-17 dihydroxyisosteviol and 16-oxime, 17-hydroxyisosteviol were top ranked molecules and might be potent molecules for the inhibition of our targets. The approach adopted in this study expedites the selection of *C. diphtheriae* putative proteins for the development of novel drugs and vaccines on broad-spectrum, owing to the fact that some of these targets have already been identified and validated in other organisms [35].

Though *C. diphtheriae* is of a large medical importance and research has been performed for more than a century, a little

is currently known about the molecular basis of pathogenicity and factors contributing to the virulence of nontoxigenic *C. diphtheriae* isolates. The advancement in OMICs sciences helped enough the mankind to identify novel therapeutic targets. Due to the emergence of drug resistance in *C. diphtheriae*, it is essential to identify new therapeutic targets for the better cure of diphtheria disease.

## Conclusion

Diphtheria is somehow forgotten disease but still for past few year cases had been reported throughout the world. This occurrence has concerned the scientific community to rethink over the currently available treatments. The availability of genomic data provides means to better understanding the molecular and genetic basis of virulence of this bacterium, enabling a detailed investigation of *C. diphtheria*. In the long run, providing a new gate way for development and/or improvement of potent vaccine.

## Acknowledgements

Syed Babar Jamal acknowledge all the authors for their contributions.

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