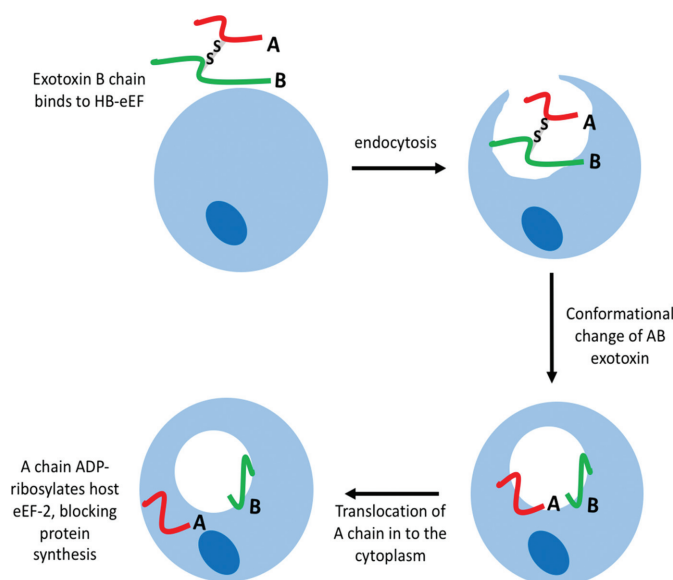


## Microbe Profile: *Corynebacterium diphtheriae* – an old foe always ready to seize opportunity

Paul A. Hoskisson\*



### Graphical abstract

Diphtheria AB toxin mode of action. The diphtheria AB exotoxin consists of two polypeptide chains – A and B which are linked by a disulphide bridge. The B chain binds to the heparin-binding epidermal growth factor precursor on eukaryotic cells and is endocytosed. Acidification of the endosome results in a conformational change to the A and B chains and breaking of the disulphide bridge. The B chain remains in the endosome, but the A chain is translocated to the cytoplasm where it ADP-ribosylates host eEF-2, blocking protein synthesis which leads to cell death.

### Abstract

*Corynebacterium diphtheriae* is a globally important Gram-positive aerobic Actinobacterium capable of causing the toxin-mediated disease, diphtheria. Diphtheria was a major cause of childhood mortality prior to the introduction of the toxoid vaccine, yet it is capable of rapid resurgence following the breakdown of healthcare provision, vaccination or displacement of people. The mechanism and treatment of toxin-mediated disease is well understood, however there are key gaps in our knowledge on the basic biology of *C. diphtheriae* particularly relating to host colonisation, the nature of asymptomatic carriage, population genomics and host adaptation.

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**Keywords:** *Corynebacterium diphtheriae*; diphtheria; toxin; exotoxin; actinobacteria.

**Abbreviation:** eEF-2, eukaryotic elongation factor 2.

## TAXONOMY

Domain *Bacteria*, phylum Actinobacteria, order Corynebacteriales, family Corynebacteriaceae, genus *Corynebacterium*, species *C. diphtheriae*. It is often further divided into four biovars (*belfanti*, *intermedius*, *gravis*, *mitis*) based on biochemical testing, however this is not well supported by genomic analysis [1].

## PROPERTIES

*C. diphtheriae* is a Gram-positive, aerobic, non-sporulating, non-capsulated and non-motile bacterium of around 2 µm in length. *C. diphtheriae* is the aetiological agent of the upper respiratory disease diphtheria and is generally considered an archetypal upper respiratory mucosal pathogen, however in recent years it has become apparent that invasive strains exist [2]. Diphtheria is a toxin-mediated disease, caused by strains lysogenized by the temperate  $\beta$ -corynephage which carry the iron-regulated diphtheria-toxin gene [3]. *C. diphtheriae* possesses a complex cell envelope architecture consisting of peptidoglycan linked to a layer arabinogalactan decorated with mycolic acids and glycolipids [4].

## GENOME

The genome of *C. diphtheriae* is around 2.45 Mbp, with a core genome of around 1632 genes and a pan-genome of around 4786 genes [5]. The genome has a G+C content of approximately 53.5% with a coding density of around 88% [6]. The differences between genomes is largely confined to the presence of prophages, transposons, restriction-modification systems and CRISPR elements. A total of 57 genomic islands have been identified and it appears that recombination plays an important role in *C. diphtheriae* genome evolution [2]. There is some variation in the genomes that relate to pathogenicity, with  $\beta$ -corynephage carriage being variable between strains and its presence does not correlate with MLST or biovar designations. Additionally, non-toxicogenic, *tox* gene-bearing strains are also found as clinical isolates. Variation is also observed in the pilli encoding *spa* operons, with at least two operons being present in all strains, but with varying numbers of pilus subunit encoding genes being found within genomes. This variation in pilus structure correlates well with the ability of *C. diphtheriae* strains to cause severe, invasive non-toxicogenic disease [5, 7].

## PHYLOGENY

The genus *Corynebacterium* forms a monophyletic family within the order Corynebacteriales [8]. The phylogenetic relationships within this group are complex and only the closely related zoonotic pathogens *C. pseudotuberculosis* and *C. ulcerans* are capable of causing diphtheria-toxin-mediated disease along with *C. diphtheriae* [9]. This suggests that *C. diphtheriae* strains may have initially emerged as a pathogen in animals prior to human host adaptation.

## KEY FEATURES AND DISCOVERIES

Toxicogenic *C. diphtheriae* is responsible for causing severe, inflammatory, toxin-mediated upper respiratory tract disease characterized by the presence of a grey layer of dead tissue termed the pseudomembrane. Initial symptoms are characterized by severe sore throats, high temperature and can often lead to death. The diphtheria AB exotoxin binds to host heparin-binding epidermal growth factor precursor. The toxin is subsequently endocytosed and processed within the cell, resulting in the ribosylation of eEF-2 causing inhibition of protein synthesis leading to cell death (see Graphical abstract). In addition to toxin-mediated disease, significant human disease can be caused by non-toxicogenic *C. diphtheriae* strains, which are now emerging as a significant cause of infections such as persistent sore throats, endocarditis, septic arthritis, osteomyelitis and cutaneous infections [2].

*C. diphtheriae* was first isolated by Loeffler [10] and was one of the first applications of 'Koch's Postulates' to confirm the aetiological agent of a disease. Prior to the introduction of the vaccine in industrialized countries in the 1940s and 1950s, diphtheria was a leading cause of child mortality. Diphtheria remains a significant health problem in countries with poor routine vaccine coverage and under-reporting of cases in certain areas suggests the burden of disease is likely to be greater than reported. The introduction of the vaccine and the subsequent global immunization initiative reduced cases to around 5000 per year globally. Major post-vaccination epidemics have included those in the 1990s in the former Soviet Union states (>157 000 cases and around 5000 deaths), notable outbreaks in Colombia, India, Norway, Nigeria, Thailand, Brazil, Laos [2] and most recently in 2017 in Indonesia, Venezuela and Bangladesh. The majority of these outbreaks can be linked to the collapse of healthcare provision due to social upheaval or the displacement of people due to armed conflict, famine, natural disasters and economic changes. This emphasizes the efficacy and importance of vaccination programmes, prior to and during outbreaks, and highlights that diphtheria can rapidly re-emerge given the right conditions with devastating effects.

The diphtheria vaccine is a toxoid composed of formaldehyde inactivated diphtheria exotoxin. The vaccine is normally delivered in combination with tetanus and acellular pertussis to children under 7 years of age, although in the UK (as of August 2017) it is now delivered as part of the 6 in 1 vaccine given at 8, 12 and 16 weeks. Treatment of diphtheria is normally with antitoxin, to neutralize the effects of circulating exotoxin along with a course of antibiotics (usually penicillin or erythromycin). Non-toxicogenic *C. diphtheriae* infections usually respond to penicillin or erythromycin treatment, with antibiotic resistance not commonly observed in *C. diphtheriae* strains.

## KEY QUESTIONS

- We have little understanding of asymptomatic carriage of non-toxicogenic *C. diphtheriae* strains as part of the

natural upper respiratory tract microbial community, yet there is potential for these strains to undergo phage conversion (to produce toxin) followed by dissemination resulting in disease outbreaks. Is this a major driver in the rapid resurgence of diphtheria in unvaccinated and/or displaced populations?

- What factors govern the colonization, persistence and invasive nature of *C. diphtheriae* in the naso-pharynx beyond the few relatively well-known virulence factors?
- Does vaccine-driven selection play a role in the emergence of non-toxigenic invasive *C. diphtheriae* strains?
- Vaccinated individuals produce antibodies to neutralize the effects of the exotoxin but it is unclear if this is sufficient to prevent colonization or eliminate non-toxigenic strains from the host.

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#### Conflicts of interest

The authors declare that there are no conflicts of interest.

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