Chapter 2
Corynebacterium diphtheriae, Corynebacterium ulcerans and *Corynebacterium pseudotuberculosis*—General Aspects

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Abstract *Corynebacterium diphtheriae*, *Corynebacterium ulcerans* and *Corynebacterium pseudotuberculosis* are potentially diphtheria toxin-producing microorganisms related to different infectious processes involving both human and animal hosts. This chapter aims to concise the current aspects concerning to the pathogenesis, epidemiology of diseases caused by those species and transmission amongst human and animal hosts. Aspects related to virulence factors, diagnosis and some molecular features observed after genome sequencing of some isolates were also approached.

Keywords *Corynebacterium diphtheriae* · *Corynebacterium ulcerans* · *Corynebacterium pseudotuberculosis* · Diphtheria toxin · Epidemiology · Pathogenicity · Virulence factors

2.1 Introduction

2.1.1 Human and Animal Infections Caused by Potentially Toxigenic Corynebacteria

Corynebacterium diphtheriae, Corynebacterium ulcerans and *Corynebacterium pseudotuberculosis* constitute a group of potentially toxigenic microorganisms related to different infectious processes involving both human and animal hosts.

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C. diphtheriae is the main causative agent of diphtheria, a toxemic disease whose prevention depends on the implementation of effective immunization programs by using toxoid molecules (Hadfield et al. 2000; Vitek and Wharton 2008). Typically, classical respiratory diphtheria presents with a swollen 'bull neck' and an adherent 'pseudomembrane' in the respiratory tract mucosa. The pseudomembrane, considered as the main pathognomonic sign of diphtheria, may extend into the trachea, bronchi and bronchioles leading to severe limitations in airflow and bronchopneumonia. By multiplying on the infection site, toxigenic strains produce diphtheria toxin (DT) that is absorbed into circulation, acting in many tissues, with particular tropism for the myocardium, central nervous system, kidney and adrenal glands. Death may result from suffocation by tough local inflammatory effects of DT on the respiratory tract, 'pseudomembranes' dislodgement or from systemic effects of DT (mainly myocarditis and renal failure) (Hadfield 2000).

Diphtheria was one of the first infectious diseases to be conquered through mass immunization with toxoid. However, the concentration of protective antibodies decreases by 10 % every year (Vitek and Wharton 1998). Over the last decades the increasing number of reported cases of atypical infections due to toxigenic and non-toxigenic *C. diphtheriae* strains in partially immunized persons also became a matter of concern (Cardénas et al. 1972; Zuber et al. 1992; Patey et al. 1995; Bitragunta et al. 2010). In many developing countries diphtheria continues to occur in children, adolescents and adults with high case-fatality rates due to inadequate nationwide coverage of immunization programs (Singh et al. 1999; Popovic et al. 2000; Mattos-Guaraldi et al. 2003; Sharma et al. 2007). Toxigenic *C. diphtheriae* may circulate in a community for 20 years after a reported case of diphtheria, even in countries where immunization programs are followed with great efficiency (De Zoysa et al. 1995; Golaz et al. 2000; Wagner et al. 2012). The introduction of toxigenic strains in a susceptible population may result in diphtheria outbreaks. All these aspects emphasize the need of vaccination strategies directed to persons of all ages and different ethnic groups and continuous surveillance of population's immunity and new diphtheria cases (Dittmann et al. 2000).

Nowadays there has been an increase in incidence of cases of infections due to non-toxigenic *C. diphtheriae*, including persistent sore throats and severe pharyngitis/tonsillitis. Invasive disease such as endocarditis, septic arthritis, splenic abscesses and osteomyelitis are not uncommon, including in cancer patients and patients making use of indwelling medical devices (Alexander 1984; Tiley et al. 1993; Lin et al. 1994; Poilane et al. 1995; Golaz et al. 2000; Mattos-Guaraldi et al. 2001; Dzupova et al. 2005; Hirata Jr. et al. 2008; Gomes et al. 2009; Farfour et al. 2012). The overall lack of information on the prevalence of colonization and disease by *C. diphtheriae* in the population may be partially due to a reduction in routine screening for this organism (Efstratiou and George 1999).

The transmission of *C. diphtheriae* is mainly from person-to-person by droplets of respiratory secretions. In endemic regions, especially in tropical areas, infections of skin lesions may serve as a reservoir for diphtheria bacilli in a variety of skin wounds, including those caused by insect bites and trauma (Werdmuller et al. 1996) as well as leishmaniotic and neoplastic ulcers (Vera et al. 2002; Mattos-Guaraldi

et al. 2003). Skin infections may be more contagious than those of the respiratory tract (MacGregor 1990; Quick et al. 2000). Although classically considered as a strict human pathogen, *C. diphtheriae* is also capable to infect animals such as cats (De Zoysa et al. 2005; Hall et al. 2010), cows (Corboz et al. 1996) and horses (Leggett et al. 2010).

Human infections by *C. ulcerans*, including diphtheria, may be fatal and usually occur in adults with close animal contact (Wellinghausen et al. 2002; Lartigue et al. 2005). More recently, the majority (approximately 75%) of the cases of zoonotic diphtheria caused by *C. ulcerans* has occurred in adult patients who had been fully or partially vaccinated with diphtheric toxoid (Dias et al. 2011). During the last decades, the frequency and severity of human infections associated with *C. ulcerans* appear to be increasing in different countries (Hatanaka et al. 2003; Dewinter et al. 2005; Bonmarin et al. 2009; Hogg et al. 2009; Komiya et al. 2010; Kimura et al. 2011). In Europe, *C. ulcerans* is currently isolated in more frequency from diphtheria cases than *C. diphtheriae* (De Zoysa et al. 2005; Perkins et al. 2010; Taylor et al. 2010; Wagner et al. 2010).

The main reservoir of *C. ulcerans* seems to be cattle, in which it may induce mastitis. Cases of infection due to *C. ulcerans* in other animals species such as macaques (Bergin et al. 2000), squirrels (Olson et al. 1988), otters (Foster et al. 2002), camels (Tejedor et al. 2000), whales and lions (Seto et al. 2008), dogs (Katsukawa et al. 2009; Dias et al. 2010), cats (De Zoysa et al. 2005), pigs (Schuhegger et al. 2009) and goats (Morris et al. 2005) have been described.

C. ulcerans may be found colonizing the nasopharynx of asymptomatic rural workers. Some cases have no association with a farming community or the consumption of raw milk products or having contact with farm animals or their waste, which suggests other routes of infection (De Zoysa et al. 2005). Recently, there has been an increased concern over transmission of *C. ulcerans* between domestic animals and humans (Dewinter et al. 2005; De Zoysa et al. 2005; Lartigue et al. 2005; Aaron et al. 2006; Seto et al. 2008; Tiwari et al. 2008; Katsukawa et al. 2009). The circulation of this pathogen in apparently healthy dogs was observed in metropolitan areas of both industrialized and developing countries. Veterinary clinics should implement guidelines and be aware of carriage of *C. ulcerans* in the throat or nares of asymptomatic animals (Katsukawa et al. 2009; Dias et al. 2010).

C. ulcerans infected patients may exhibit skin lesions that completely mimic cutaneous diphtheria or present as a tracheal-bronchial tree covered by pseudomembranes (Wagner et al. 2001; Dewinter et al. 2005; Mattos-Guaraldi et al. 2008; Wagner et al. 2010). Independent of DT production, *C. ulcerans* was also found to produce clinical syndromes of the lower respiratory tract, such as pneumonia (Hommez et al. 1999; Hatanaka et al. 2003), pulmonary granulomatous nodules (Dessau et al. 1995), occasionally associated with signs of systemic inflammatory response syndrome and disseminated intravascular coagulation (Nureki et al. 2007). *C. ulcerans* infections may occur in children previously immunized against diphtheria (Leek et al. 1990). Older urban adults may be also at risk for toxic complications due to inadequate immune status (Gubler et al. 1990; Wellinghausen et al. 2002).

C. pseudotuberculosis is the etiological agent of caseous lymphadenitis (CLA) in small ruminant populations, such as sheep and goats that sometimes presents as pneumonia, hepatitis, pericarditis, mastitis, arthritis and subcutaneous abscesses. The pathogen is also associated with lymphadenitis in horses, ulcerative lymphangitis and pigeon fever in cattle, camels, swine, elks and buffaloes (Selim 2001; Foley et al. 2004, Perkins et al. 2004; Baird and Fontaine 2007; Sharpe et al. 2010; Kelly et al. 2012). Though the pathogen is distributed worldwide, it has the most serious economic impact in Oceania, Africa and South America (Estevao et al. 2007; Komala et al. 2008; Tarello and Theneyan 2008; Stefanska et al. 2008; Seyffert et al. 2010). The increase in number of infections could be the result of reporting bias, environmental factors facilitating infection or host factors such as greater herd susceptibility (Foley et al. 2004). Similar to diphtheria in humans, clonally expanding epidemic of animal infection caused by *C. pseudotuberculosis* may also occur (Kombarova et al. 2001; Saikya et al. 2010).

The potential of *C. pseudotuberculosis* to survive for several weeks in the environment likely contributes to its ability to spread within a herd or flock (Augustine and Renshaw 1986; Yeruham et al. 2004). Transmission among sheep or goats occurs mainly through contamination of superficial wounds, which can appear during common procedures, such as shearing, castration and ear tagging or through injuries of the animals' bodies generated by other traumatic events. Not infrequently, contaminated sheep cough bacteria onto skin cuts of other sheep, constituting another means of transmission (Paton et al. 1995; Williamson 2001).

Though transmission of *C. pseudotuberculosis* still needs to be determined with certainty, in cattle as well as in buffaloes the microorganism may be vectored to animals by flies such as *Musca domestica* and *Hippobosca equina*. In cases of edematous skin disease (OSD) in buffaloes the pathogen may be identified in up to 20% of flies in the vicinity of diseased animals (Yeruham et al. 1996; Braverman et al. 1999; Selim 2001; Spier et al. 2004). In OSD cases, *C. pseudotuberculosis* biovar *equi* exert their pathogenesis by secretion of DT in addition to phospholipase D (PLD) and their lipid contents of the cell walls (Selim 2001).

Very few studies have indicated the isolation of the causal agent of CLA and OSD from humans. Human infections caused by *C. pseudotuberculosis* are frequently similar to that observed in sheep and goats (CLA) and usually need the excision of infected lymph nodes accompanied of supplementary antimicrobial treatment, without observation of toxemic manifestations. The microorganism is usually acquired after close contact with infected animals, and no underlying diseases or predisposing conditions have been observed in infected patients (Hemond et al. 2009; Join-Lambert et al. 2006; Peel et al. 1997; Romero-Perez et al. 2004). Most of the reported cases have been related to occupational exposure, ingestion of raw goat meat and cow milk. About 25 cases of infection of humans with this microorganism have been reported in the literature (Mills et al. 1997; Peel et al. 1997; Liu et al. 2005). Peel and co-workers (1997) reviewed 22 cases, in which infected humans generally presented with lymphadenitis, abscesses and constitutional symptoms. Liu and co-workers (2005) reported a *C. pseudotuberculosis* infection in a patient's eye, due to an ocular implant. In most cases, the patients received

antibiotic therapy and the affected lymph nodes were surgically removed. One case with toxemic symptoms from an injecting drug patient with endocarditis was recently reported. The patient had no history of animal contact and no possible source for *C. pseudotuberculosis* infection was identified (Wagner et al. 2011).

2.2 Microbiological and Diagnosis Aspects

Corynebacterium species constitute a group of catalase-positive, non-motile and non-spore forming, pleomorphic Gram-positive rods. The club or bar appearance of the cells is due to stored inorganic phosphate which forms metachromatic granules when stained by especial techniques (Funke and Bernard 2011). However, many *C. diphtheriae, C. ulcerans* and *C. pseudotuberculosis* strains may present confusing coccobacillary arrangements lacking phosphate granules. Fermentation of carbohydrates is diverse though the species are capable to ferment glucose and maltose (Table 2.1). Production of acid from sucrose may also be observed, including for some *C. diphtheriae* strains (Hirata Jr. et al. 2011). *C. diphtheriae* subspecies differing slightly in their colonial morphology and biochemical properties are currently recognized: *gravis*, *intermedius*, *mitis* and *belfanti*. Two biovars of *C. pseudotuberculosis*, based on nitrate-reducing ability, have been reported: nitrate-negative are referred as biovar *ovis* (usually isolated from goats and sheep) and nitrate-positive as biovar *equi* (usually isolated from horses and cattle) (Dorella et al. 2006). The strains isolated from cattle are variable for nitrate reduction (Tejedor-Junco et al. 2008). *C. pseudotuberculosis* and *C. ulcerans* are capable to produce urease that may distinguish these species from *C. diphtheriae*, while *C. ulcerans* and *C. diphtheriae* are capable to produce DNase (Cetinkaya et al. 2002; Pimenta et al. 2008b; Dias et al. 2011).

C. diphtheriae, *C. ulcerans* and *C. pseudotuberculosis* are cystinase positive in Tinsdale agar medium and negative for the pirazinamidase (PYZ) test. When suspect organisms are inoculated transversally with a hemolytic *Staphylococcus aureus* strain, reverse CAMP reactions in Blood Agar Medium are observed for *C. ulcerans* and *C. pseudotuberculosis* due to the ability to produce PLD. These phenotypic aspects are conventionally used in laboratory diagnosis of potentially toxigenic species (Funke and Bernard 2011).

Diphtheria is no longer diagnosed easily on clinical grounds. Mild cases of diphtheria resemble pharyngitis and pseudomembrane may be absent. In addition to cause invasive diseases non-toxigenic *C. diphtheriae* strains have the potential to undergo lysogenic conversion and to produce DT *in vivo*. Therefore, diagnosis of diphtheria is a laboratory emergency and deserves priority. An important test in the microbiological diagnosis of diphtheria is the detection of DT producing strains. Toxigenicity tests are not readily available in most diagnostic laboratories; it is strongly recommended that all isolates be referred promptly to reference laboratories, which are proficient in performing these tests. Several *in vitro* methods are available, including the conventional Elek test, modified Elek tests and genotypic

Table 2.1 Phenotypic properties of potentially toxigenic corynebacteria

Species	Conventional phenotypic tests											
	Hemolysis	DNase	Cystinase (H_2S)[b]	Pirazina-midase	Nitrate reduction	Urea hydrolysis	Glucose	Maltose	Sucrose	Glycogen	Starch	CAMP reaction
C. diphtheriae[a]												
Subsp. gravis	−	+	+	−	+	−	+	+	−/+	+	+	−
Subsp. mitis	+/−	+	+	−	+	−	+	+	−/+	−	−	−
Subsp. intermedius[c]	−	+	+	−	+	−	+	+	−/+	−	−	−
Subsp. belfanti	−	+	+	−	−	−	+	+	−/+	−	−	−
C. ulcerans[a]	+	+	+	−	−/+	+	+	+	−/+	+/−	+	Rev
C.pseudotuberculosis												
Biovar ovis	+	−	+	−	−	+	+	+	−/+	−/+	+	Rev
Biovar equi	+	−	+	−	+	+	+	+	−/+	−/+	+	Rev

[a] Species determined by API Coryne System

[b] Cystinase activity determined in Tinsdale agar medium or PISU test

[c] Lipophilic subspecies

Rev reverse CAMP test observed on Blood agar medium using hemolytic *S. aureus* strain

tests based on PCR for detection of *tox* gene. The gold standard assay is the *in vitro* Vero cell assay, which is based on the cytotoxicity of diphtheria toxin to cultured Vero cells (Farizo et al. 1993; Hauser et al. 1993; Pallen et al. 1994; Nakao and Popovic 1997; Efstratiou et al. 1998, 1999, 2000; Mothershed et al. 2002; Pimenta et al. 2008a; Konrad et al. 2010).

Rapid and accurate detection of potentially toxigenic corynebacteria is necessary for the determination of appropriate measures in controlling the dissemination of toxigenic clones in both veterinary and/or human populations. PCR assays offer many advantages over standard phenotypic tests: they are simple, easy to interprete, and becoming more widely available in laboratories in both industrialized and in developing countries. Recently, a multiplex PCR was proposed for laboratory diagnosis of *C. diphtheriae*, *C. ulcerans*, and *C. pseudotuberculosis*, with a preparation of primers capable to discriminate toxigenic isolates of the three species. The reaction mixture consists of primers (reverse and forward) for amplification of *rpoB* (for corynebacteria); *16SrDNA* (for *C. ulcerans* and *C. pseudotuberculosis*), *dtxR* (for *C. diphtheriae*), *pld* (specific for *C. pseudotuberculosis*), and *dipht4* (for *tox* gene) (Torres et al. 2013).

2.3 Antimicrobial Susceptibility

Antimicrobial treatment in diphtheria cases is capable to both eradicate the pathogen and limit the production of DT by toxigenic strains, alongside to reduce the risk of transmission of microorganisms by symptomatic carriers. Though important for the treatment of infections caused by *C. diphtheriae*, the antimicrobial treatment does not substitute the immunotherapy with diphtheria antitoxin in infections caused by toxigenic strains. *C. diphtheriae* strains are typically susceptible to beta-lactam antibiotics (especially Penicillin G and amoxicillin) and erythromycin. However, tolerance or resistance to penicillin and/or to erythromycin was reported in different countries (Coyle et al. 1979; Farizo et al. 1993; Maple 1994; Patey et al. 1995; von Hunolstein et al. 2002; Zasada et al. 2010; Pereira et al. 2008).

Resistance to erythromycin was reported to be carried by a plasmid and supposed to be associated to coryneform microorganisms colonizing the skin (Schiller et al. 1983; Serwold-Davis and Groman 1986). Multidrug-resistant (MDR) *C. diphtheriae* strains have been also described in recent global literature (Pereira et al. 2008; Zasada et al. 2010; Mina et al. 2011).

The recommendation for the treatment of diphtheria caused by *C. ulcerans* is the administration of antimicrobials penicillin G or erythromycin, in addition to the administration of diphtheria antitoxin. The resistance of *C. ulcerans* to antimicrobial treatment with penicillin in a dog has been reported (Lartigue et al. 2005), as well as the resistance or decreased sensitivity to clindamycin (Katsukawa et al. 2012). The resistance to erythromycin was observed in a *C. ulcerans* strain isolated from a fatal case of diphtheria, and the determination of antimicrobial resistance was highlighted (Tiwari et al. 2008).

C. pseudotuberculosis is generally susceptible to many antibiotics used for the treatment of corynebacterial infections, including penicillin G, ampicillin, erythromycin, gentamicin, and sulfamethoxazole-trimethoprim. However, in cases of lymphadenitis, antimicrobial treatment and surgical approaches to remove the infected lymph nodes are necessary, since antimicrobials do not reach inhibitory concentrations inside the caseous lesions (Dorella et al. 2006).

2.4 Virulence Factors

Diphtheria toxin The major known virulence factor of *C. diphtheriae* is the DT (Pappenheimer 1993; Wang and London 2009). The *tox* gene for DT production is present in corynebacteriophages (β tox^+, γ tox^+, ω tox^+), capable to integrate into the chromosomes of *C. diphtheriae*, *C. ulcerans* and *C. pseudotuberculosis*. The expression of DT is also dependent on chromosomal genes. In low intracellular iron concentrations, the regulatory *dtxR* gene is inhibited, resulting in an increase on DT production by *C. diphtheriae* strains.

DT is a polypeptide of 535 amino acids with a molecular weight of approximately 62,000 Da, composed of two fragments, A (DT-A—with the domain A (enzymatic active)) and B (DT-B—with domains B (binding to the cell receptor: HB-EGF (heparin binding epidermal growth factor) precursor along with DRAP27/CD9 membrane proteins), and T (translocation of the active fragment across the membrane). DT-A and DT-B are linked by disulfide bonds. DT-A is enzymatically active while DT-B, although not toxic, is essential for penetration of TDA in the cell cytoplasm (Funke et al. 1997; D'Silva and Lala 2000). DT has a lower minimum lethal dose of 50–100 ng/kg body weight (Pappenheimer 1977, 1993; Mekada et al. 1988). A single molecule of DT-A, when introduced directly into the cytoplasm, is sufficient to kill the eukaryotic cell (Yamaizumi et al. 1978). Besides the ability to inhibit the protein synthesis (Chang et al. 1989a), DT is also capable to induce internucleossomal cleavage of DNA (indicative of apoptosis-inducing activity) assigned to DT-A (Chang et al. 1989a, b; Nakamura and Wisnieski 1990), process that precedes cytolysis and does not seem to be due to the inhibition of protein synthesis.

The efficacy of diphtheric toxoid vaccine or antiserum against the zoonotic diphtheria caused by *C. ulcerans* still remains unknown. Differences in nucleotide sequences of the *tox* genes may occur among *C. diphtheriae* strains as well as among *C. ulcerans* strains (Mekada et al. 1988; Bitragunta et al. 2010). These facts may contribute towards situations in which individuals vaccinated with diphtheric toxoid or undergoing serum therapy may not present full protection against infections caused by toxigenic corynebacteria. Even if it is considered that the diphtheric toxoid may have a protective effect against diphtheria caused by *C. ulcerans* (i.e. through the presence of attenuated clinical symptoms), it should be remembered that the vaccination only prevents the action of DT and probably does not impede colonization by toxigenic corynebacteria (Neal and Efstratiou 2007; Begue 2010).

Since there are still many issues that require better assessment, not only in relation to clinical-laboratory diagnosis, but also in relation to treating and preventing diseases caused by potentially toxigenic corynebacteria, a better comprehension of virulence factors other than DT production is necessary.

Toxins other than DT PLD exotoxin is considered to be the major virulence factor expressed by *C. ulcerans* and *C. pseudotuberculosis* strains (Lipsky et al. 1982; Hodgson et al. 1992; Dorella et al. 2006). Endowed of a sphingomyelinase activity, PLD toxin is expressed from a chromosomal gene present in *C. ulcerans* and *C. pseudotuberculosis*. PLD is capable to induce the vascular permeability possibly contributing to the spread of the bacteria from the initial site of infection to secondary sites (Carne and Onon 1978; Lipsky et al. 1982; Coyle and Lipsky 1990; McNamara et al. 1995; Tachedjian et al. 1995; Peel et al. 1997; Tambourgi et al. 2002; Dorella et al. 2006). Cytotoxic effects and death of caprine macrophages due to action of PLD was observed during infection with *C. pseudotuberculosis* (Tashjian and Campbell 1983). Expression of PLD is regulated by multiple environmental factors, including cell-density and heat shock (McKean et al. 2007). The use of a PLD antitoxin may prevent the systemic dissemination of *C. pseudotuberculosis* but not the development of abscesses (Williamson 2001).

A putative *C. pseudotuberculosis* iron uptake gene cluster has a role in its virulence. The four genes in this putative operon were identified downstream from the *pld* gene. They were designated as Fe acquisition genes—*fagA*, *fagB*, *fagC* and *fagD*. Since *C. pseudotuberculosis* is an intracellular pathogen, this bacterium must be able to acquire iron from an environment in which this nutrient is scarce. Although there was no alteration in the utilization of iron by a *fagB(C)* mutant *in vitro*, this mutant had a decreased ability to survive and to cause abscesses in experimentally-infected goats (Billington et al. 2002).

In human infections caused by *C. ulcerans*, the occurrence of necrosis and mucosal ulceration as well as other clinical manifestations in the lower respiratory tract were attributed to the production of both DT and PLD (Dessau et al. 1995; Seto et al. 2008). The genome sequencing investigations of two Brazilian *C. ulcerans* isolates showed the presence of *pld* gene and absence of *tox* gene in both 809 strain (isolated from a case of human fatal pneumonia) and BR-AD22 strain (isolated from an asymptomatic dog) (Trost et al. 2011).

C. pseudotuberculosis and *C. ulcerans* strains also showed colonization behavior in the *Caenorhabditis elegans* and induction of death in *Galleria mellonella* models. Interestingly, a *C. ulcerans* PLD-deficient strain showed unaltered colonization behavior compared to the parental strain BR-AD22 in the *C. elegans*, although it caused a less severe response in the *Galleria* model. The strongest effects on the *Galleria* larvae obtained for *C. pseudotuberculosis* and *C. ulcerans* strains, which exhibited high degrees of melanisation, immobility and rapid death, were attributed to PLD production (Ott et al. 2012). Meanwhile, the PLD-positive 809 human isolate was considered more virulent than the non-arthritogenic PLD-positive BR-AD22 strain, since it was capable to induce arthritis and to cause mice death (Dias et al. 2011).

Adhesion to biotic and abiotic surfaces During the last decades the current reporting of diphtheria outbreaks attracted justifiable attention and stimulated the search for microbial factors responsible for invasiveness of *C. diphtheriae* and *C. ulcerans*. Changes in the circulating strains of *C. diphtheriae* could be responsible for the episodic diphtheria epidemic waves. However, the microbial factors that distinguish epidemic from non-epidemic *C. diphtheriae* strains remain under investigation (Popovic et al. 1996; Funke et al. 1999). Other factors besides antitoxin protection influence vulnerability to diphtheria, namely, the general immune status of the person infected, as well as the number and virulence of diphtheria bacilli involved. Moreover, immunized patients may have *C. diphtheriae* bacteremia and endocarditis in the absence of characteristic toxin-mediated lesions, confirming that invasive and toxigenic properties are independent of each other (Mattos-Guaraldi and Formiga 1998; Hogg et al. 1996; Patey et al. 1997; Funke et al. 1999). Multilocus sequence typing (MLST) to study genetic relationships reinforced the fact that invasive strains may be found in different clonal complexes of *C. diphtheriae* (Viguetti et al. 2012).

In the 1980s, opening studies started dealing with the adhesive properties of the *C. diphtheriae*. The multifactorial nature of the adhesiveness was considered once *C. diphtheriae* strains adhered to several biotic and abiotic substrates in varied intensities. Bacterial adhesive properties were independent of toxin production. Studies using human and animal erythrocytes showed *C. diphtheriae* capable to adhere differentially according to sucrose fermenting biotypes and subspecies (*gravis*, *mitis*, *intermedius* and *belfanti*) (Yanagawa and Honda 1976; Deacock et al. 1983; Kostyukova and Pereverzev 1985; Mattos-Guaraldi and Formiga 1986; Karas et al. 1991; Mattos-Guaraldi and Formiga 1991; Kostyukova and Karas 1991; Kostyukova et al. 1992; Mattos-Guaraldi and Formiga 1992; Mattos-Guaraldi and Formiga 1998a, b; Mattos-Guaraldi et al. 1999a, b; Hladka and Motyka 1998; Colombo et al. 2001). Further studies demonstrated that non-toxigenic (Bertuccini et al. 2004) and toxigenic *C. diphtheriae* strains have the ability to interact with human epithelial cells (Hirata Jr. et al. 2002, 2004). The internalization of *C. diphtheriae* by non-professional phagocyte cells was then documented. Invasive *C. diphtheriae* strains were able to survive within human epithelial cells (Hirata Jr. et al. 2002; Bertuccini et al. 2004) at different levels. *C. diphtheriae* strains were also capable to adhere to U937 macrophages inducing both necrosis and apoptosis. Both activities were independent to the expression of DT, since the non-toxigenic ATCC 27010 also induced both apoptosis and necrosis (Santos et al. 2010).

The surface lipid components of *C. pseudotuberculosis* may contribute to the pathogenesis, since it can induce hemorrhagic necrosis after intradermal injection in guinea pigs (Carne et al. 1956; Jolly 1965, 1966; Hard 1972, 1975). Additionally, mouse peritoneal macrophages were highly susceptible to the necrotizing/cytotoxic effect of *C. pseudotuberculosis* lipids, whereas rabbit cells were shown to be resistant (Hard 1975). However the molecules that permit the microbial interaction to animal and human cells are still unknown for *C. pseudotuberculosis* and *C. ulcerans*.

Few data are available dealing with carbohydrate structures on the cell surfaces of potentially toxigenic corynebacteria. *C. diphtheriae* strains were shown to

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