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Sarela García-Masedo Fernández [a,*], Julio García [a], Juan Antonio Sáez-Nieto [a,b] y Beatriz Orden [a]

[a] *Servicio de Microbiología, Hospital Universitario Puerta de Hierro Majadahonda, Majadahonda (Madrid), España*
[b] *Servicio de Microbiología, Instituto de Salud Carlos III, Majadahonda (Madrid), España*

* Autor para correspondencia.
Correo electrónico: sarelagmf@gmail.com
(S. García-Masedo Fernández).

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Skin infection by *Corynebacterium diphtheriae* and *Streptococcus pyogenes*: an unusual association

Infección cutánea por Corynebacterium diphtheriae *y* Streptococcus pyogenes*: una asociación inusual*

Dear Editor:

Corynebacterium diphtheriae is a noncapsulated, club-shaped facultative anaerobic Gram-positive bacilli. Opportunistic or cutaneous co-infection caused by this microorganism, especially non-toxigenic strains, has become important in travellers[1]. The skin lesions are generally ulcerative with a torpid and nonspecific evolution, which usually appear after a bite or minor trauma[2]. These infections have a low incidence[3], which is why this microorganism is often not considered as the first etiological diagnosis, so in many of the cases can be unnoticed. A study of two cases of infection by *C. diphtheriae* and *Streptococcus pyogenes* was performed. The microorganisms were isolated from swabs of wound exudates and were identified by mass spectrometry (MALDI-TOF MS, Bruker©) and were confirmed with the amplification and sequencing of the 16S rRNA gene. Diphtheria toxin was performed by PCR[4].

Case 1

A 28-year-old man with a recent travel history to Philippines attended for an incised wound on the back of the left foot of 15 days of evolution, with signs of cellulitis. The case was oriented as cellulitis and started intravenous treatment with ceftriaxone 1 g for 5 days and linezolid 600 mg for 3 days, after that the treatment were change to oral azithromycin for one week. In culture, *S. pyogenes* and *C.diphtheriae* were isolated. Antibiotic susceptibility testing (AST) was performed and both microorganisms were susceptible to penicillin and erythromycin. Diphtheria toxin was negative. The patients evolving favourably and subsequently decided to administer a booster of diphtheria vaccine.

Case 2

A 32-year-old man, with a recent travel history to Southeast Asia for 2 months. Attended for a traumatic wound in the heel and erythematous and crusted lesions of 2-3 cm in the right leg. Physical examination reveals a peripheral pustule with inflammation of an inguinal node without signs of cellulitis in the peripheral skin. The case was oriented as skin infection by biting of overinfected arthropods. Serology was requested for Dengue, Chikungunya and culture. *S. pyogenes* and *C.diphtheriae* were isolated. AST was performed and both microorganisms were susceptible to penicillin and erythromycin. Serologies for Dengue and Chikungunya were negative. Treatment with oral erythromycin 500 mg every six hour for 14 days was started, contact study was carried out and reinforcement of the diphtheria vaccine was administered. Diphtheria toxin was negative; the patient was evaluated for 2 weeks, showing resolution of both traumatic wound and satellite lesions.

Cutaneous infection by *C. diphtheriae* is uncommon, tends to be of torpid evolution and produce nonspecific lesions, so clinical suspicion is low. In recent years this infection has been linked mainly with travellers to endemic areas including Southeast Asia, some countries such as Cambodia, India, Indonesia, Malaysia, New Guinea, Philippines, Thailand, Brazil and others[5,6]. A study in Vancouver reports 37 cases of cutaneous diphtheria for non-toxigenic strains [8] which demonstrates the high distribution of these strains. In Europe, the data was based mainly on patients with a recent travel history[7], except in some Eastern European countries, which are considered an endemics areas[2].

Other risk factors for the infection included population with low socioeconomic resources, alcohol abuse, drugs, HIV infection, hepatitis, cirrhosis[8,3], Identification of Gram positive bacilli colonies may be considered in some cases as non-pathogenic microbiota by the genus of *Corynebacterium*, and presence of *C.diphtheriae* may be misidentified. In these cases we can apply the MALDI-TOF MS, it's an easy technique and effective cost[2].

Co-infection is a common clinical presentation. *S. pyogenes, Staphylococcus aureus*, methicillin-resistant *S. aureus, Arcanobacterium haemolyticum* and species of coagulase-negative staphylococci [8] are the more frequently association. In 2016 a third case of cutaneous diphtheria was also reported where colonies of *A. haemolyticum* were also isolated in a 50-year-old patient with a recent travel history to Guinea Bissau and mimicking pyoderma gangrenosum[9].

Benzylpenicillin and macrolides were considered first line treatment in cases of diphtheria, but in 2015 the first case of *C. diphtheriae* resistant to penicillin was published in a cutaneous infection by a non-toxigenic strain in the United Kingdom [10] However, benzylpenicilin continue to be the first option for treatment in case of diphtheria. In our cases the both strains and both *S.pyogenes* were susceptible to penicillin and erythromycin. In Spain in 2015, the first case of diphtheria was reported since 1986, in a 6-year-old unvaccinated child, who progressed unfavourably and died after one month of medical treatment. However, in relation to cutaneous diphtheria, no previous reports have been found.

The number of travellers continues to increase in Spain and Europe, which can increase the incidence of these mixed infections. The recent travel history should be recognized as an epidemiological data to highlight not only the clinical diagnosis but also the microbiological one.

Ethical approval

Not necessary.

Informed consent

Not necessary.

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M. Fernandez-Pittol [a,*], J. Bosch [a], J. Muñoz [b], M. Bodro [c]

[a] *Department of Microbiology, Hospital Clínic, Universitat de Barcelona, ISGLOBAL, Barcelona, Spain*
[b] *Department International Health, Hospital Clinic, Barcelona, Spain*
[c] *Department of Infectious Diseases, Hospital Clinic, Barcelona, Spain*

* Corresponding author. Hospital Clinic de Barcelona, Villarroel, 170, 08036 Barcelona - SPAIN. Tel.: +34.654.966.069.
E-mail address: mjfernandez@clinic.cat (M. Fernandez-Pittol).

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Diagnóstico diferencial de *Bordetella bronchiseptica* por RT-PCR en un niño con tos paroxística sin antecedentes patológicos previos

Differential diagnosis by RT-PCR of Bordetella bronchiseptica *in a child without previous pathologic antecedents suffering whooping cough*

El género *Bordetella* incluye varias especies que pueden afectar al ser humano[1]. Entre ellas destaca por su relevancia clínico-epidemiológica *B. pertussis*[2]. Sin embargo, otras especies como *B. parapertussis*, *B. holmesii*[1], y en ocasiones, *B. bronchiseptica*[3,4] pueden originar cuadros pertusoides similares.

El objetivo del presente estudio fue describir un caso de infección por *B. bronchiseptica*, y la estrategia empleada para su diagnóstico microbiológico. Varón de 21 meses, sin historial clínico de enfermedades respiratorias crónicas o alteraciones inmunológicas, que acudió a urgencias con tos paroxística y emetizante de 9 días de evolución, sin apnea, pero acompañada de estridor. El paciente se encontraba correctamente inmunizado para su edad frente a tos ferina, con 3 dosis de vacuna antidiftérica, antitetánica, antipertusis acelular administradas a los 2, 4 y 11 meses. La familia convivía con un perro que no había presentado indicios de enfermedad.

Ante este cuadro compatible con un síndrome pertusoide, se obtuvieron muestras de lavado nasofaríngeo, y se pautó una dosis inicial de azitromicina a 10 mg/kg/día seguida de un tratamiento domiciliario con 5 mg/kg/día durante 4 días. El paciente evolucionó favorablemente, sin complicaciones. La muestra de lavado nasofaríngeo se estudió mediante una técnica comercial de reacción en cadena de la polimerasa en tiempo real (RT-PCR) múltiple (Smart Bp/Bpp, Cepheid AB, Suecia), basada en la detección de las secuencias de inserción IS*481* e IS*1001*. De acuerdo a las instrucciones del fabricante, los resultados fueron presuntivamente clasificados como positivos tanto para *B. pertussis* como para *B. parapertussis*. La muestra fue posteriormente procesada empleando 5 ensayos independientes de RT-PCR frente a diferentes marcadores: IS*481*, IS*1001*, región promotora del gen de la toxina pertusis (BPTP), gen de una proteína porina de *B. pertussis* (BPTD_0837) y la secuencia de inserción similar a la IS*1001* de *B. holmesii* (hIS*1001*). Los resultados obtenidos (IS*481* confirmado como positivo, IS*1001* confirmado como positivo, BPTP positivo, BPTD_0837 negativo y hIS*1001* negativo) aportaron una identificaron como *B. bronchiseptica* de acuerdo al algoritmo descrito en la tabla 1.

Si bien *B. bronchiseptica* crece en medios habituales como agar MacConkey, el cultivo de *B. pertussis* es problemático y carece de sensibilidad. Por este motivo en la actualidad el diagnóstico de tos ferina se basa en técnicas de PCR sobre muestras nasofaríngeas, utilizando varias dianas como los segmentos de inserción IS*481* e IS*1001*, empleadas para identificar como probables a *B. pertussis* y *B. parapertussis*, respectivamente[5,6]. No obstante, estas secuencias no son absolutamente específicas de estas especies[6]. La secuencia IS*481* puede hallarse junto con *B. pertussis* en *B. holmesii* y en algunas cepas de *B. bronchiseptica*[6]. Por su parte la secuencia IS*1001* puede estar presente, además de en *B. parapertussis*, en *B. bronchiseptica*[6]. Esta secuencia muestra también gran homología con hIS*1001*, presente en *B. holmesii*[5]. Un resultado de PCR simultáneamente positivo para IS*481* y S*1001* podría inicialmente considerarse una coinfección por diferentes especies de *Bordetella*[6]. En estas situaciones, el uso de otras dianas de amplificación puede aclarar el diagnóstico. Tanto *B. pertussis* como *B. bronchiseptica* son positivas para BPTP[7]. Sin embargo, el gen BPTD_0837 es específico de *B. pertussis* y no muestra reactividad cruzada con otras especies

Tabla 1
Algoritmo de los ensayos RT-PCR multimarcador para la identificación de las principales especies de *Bordetella* spp.

Interpretación	Resultado de los ensayos				
	IS*482*	BPTP	BPTD_0837	IS*1001*	hIS*1001*
B. pertussis	+	+	+	–	–
B. parapertussis	–	–	–	+	–
B. holmesii	+	–	–	–	+
B. bronchiseptica	±	+	–	±	–