3. Bianco G, Boattini M, Audisio E, Cavallo R, Costa C. Septic shock due to meropenem- and colistin-resistant *Cupriavidus pauculus*. J Hosp Infect. 2018;99:364–5.
4. Balada-Llasat JM, Elkins C, Swyers L, Bannerman T, Pancholi P. Pseudo-outbreak of *Cupriavidus pauculus* infection at an outpatient clinic related to rinsing culturette swabs in tap water. J Clin Microbiol. 2010;48:2645–7.
5. Stovall SH, Wisdom C, Mckamie W, Ware W, Dedman H, Fisher RT. Nosocomial transmission of *Cupriavidus pauculus* during extracorporeal membrane oxygenation. ASAIO J. 2010;56:486–7.
6. Azcona-Gutierrez JM, Buendía-Moreno B, Sáez-Nieto JA, López-Brea Calvo M. Aislamiento de *Cupriavidus pauculus* en la unidad de cuidados intensivos. Enferm Infecc Microbiol Clin. 2008;26:397–8.
7. Vay C, García S, Alperovich G, Almuzara M, Lasala MB, Famiglietti A. Bacteremia due to *Cupriavidus pauculus* (formerly CDC Group IVc-2) in a hemodialysis patient. Clin Microbiol Newslett. 2007;4:30–2.

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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.

References

1. Orouji A, Kiewert A, Filser T, Goerdt S, Peitsch WK. Cutaneous diphtheria in a German man with travel history. Acta Derm Venereol. 2012;92:179–80, http://dx.doi.org/10.2340/00015555-1216
2. Jakovljev A, Steinbakk M, Mengshoel AT, Sagvik E, Br??gger-Synnes P, Sakshaug T, et al. Imported toxigenic cutaneous diphtheria in a young male returning from mozambique to Norway, March 2014. Eurosurveillance. 2014;19:1–4.
3. Billard-Pomares T, Rouyer C, Walewski V, Badell-Ocando E, Dumas M, Zumelzu C, et al. Diagnosis in France of a non-toxigenic tox gene-bearing strain of corynebacterium diphtheriae in a young male back from senegal. Open Forum Infect Dis. 2017;4:1–3, http://dx.doi.org/10.1093/oid/ofw271
4. Zakikhany K, Neal S, Efstratiou A. Emergence and molecular characterisation of non-toxigenic tox gene-bearing Corynebacterium diphtheriae biovar mitis in the United Kingdom, 2003-2012. Euro Surveill. 2014:19. Available: http://www.ncbi.nlm.nih.gov/pubmed/24925458.
5. Rahim NRA, Koehler AP, Shaw DD, Graham CR. Toxigenic cutaneous diphtheria in a returned traveller. Commun Dis Intell. 2014;38:E298–300.
6. FitzGerald RP, Rosser AJ, Perera DN. Non-toxigenic penicillin-resistant cutaneous C. diphtheriae infection: A case report and review of the literature. J Infect Public Health. King Saud Bin Abdulaziz University for Health Sciences;. 2015;8:98–100, http://dx.doi.org/10.1016/j.jiph.2014.05.006
7. Adler NR, Mahony A, Friedman ND. Diphtheria: Forgotten, but not gone. Intern Med J. 2013;43:206–10, http://dx.doi.org/10.1111/imj.12049
8. Lowe CF, Bernard KA, Romney MG. Cutaneous diphtheria in the urban poor population of Vancouver, British Columbia, Canada: A 10-year review. J Clin Microbiol. 2011;49:2664–6, http://dx.doi.org/10.1128/JCM. 00362-11
9. Morgado-Carrasco D, Riquelme-Mc Loughlin C, Fustá-Novell X, Fernandez-Pittol MJ, Bosch J, Mascaró Jr JM. Cutaneous Diphtheria Mimicking Pyoderma Gangrenosum. JAMA Dermatology. 2018;154:227-8.
10. Nelson TG, Mitchell CD, Sega-Hall GM, Porter RJ. Cutaneous ulcers in a returning traveller: A rare case of imported diphtheria in the UK. Clin Exp Dermatol. 2016;41:57–9, http://dx.doi.org/10.1111/ced.12763

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

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

The genus *Bordetella* includes various species that can affect human beings.[1] Among them, *B. pertussis* stands out for its clinical-epidemiological relevance.[2] However, other species such as *B. parapertussis*, *B. holmesii*[1] and, occasionally, *B. bronchiseptica*[3,4] can cause similar whooping cough symptoms.

The objective of this study was to describe a case of *B. bronchiseptica* infection and the strategy used for its microbiological diagnosis. A 21-month-old male, with no clinical history of chronic respiratory diseases or immunological abnormalities, who attended the emergency department with a paroxysmal cough accompanied by vomiting, which had started nine days previously, with no apnoea, but accompanied by stridor. The patient was correctly immunised for his age against whooping cough, with three doses of diphtheria vaccine, tetanus vaccine and acellular pertussis vaccine administered at 2, 4 and 11 months. The family lived with a dog that had not shown signs of disease.

In light of these symptoms compatible with whooping cough, nasopharyngeal lavage samples were obtained, and an initial dose of azithromycin at 10 mg/kg/day followed by home treatment with 5 mg/kg/day for four days was prescribed. The patient progressed favourably, with no complications. The sample of nasopharyngeal lavage was studied using a commercial multiplex real-time polymerase chain reaction (RT-PCR) technique (Smart Bp/Bpp, Cepheid AB, Sweden), based on the detection of the insertion sequences IS*481* and IS*1001*. According to the manufacturer's instructions, the results were classified presumptively as positive for both *B. pertussis* and *B. parapertussis*. The sample was subsequently processed using five independent RT-PCR assays against different markers: IS*481*, IS*1001*, promoter region of the pertussis toxin gene (BPTP), *B. pertussis* porin protein gene (BPTD_0837) and the insertion sequence similar to IS*1001* of *B. holmesii* (hIS*1001*). The results obtained (IS*481* confirmed as positive, IS*1001* confirmed as positive, BPTP positive, BPTD_0837 negative and hIS*1001* negative) contributed to an identification as *B. bronchiseptica* in accordance with the algorithm described in Table 1.

While *B. bronchiseptica* grows in common media such as MacConkey agar, the culture of *B. pertussis* is problematic and lacks sensitivity. For this reason, the diagnosis of whooping cough is currently based on PCR techniques on nasopharyngeal samples, using several targets such as the insertion segments IS*481* and IS*1001*, used to identify *B. pertussis* and *B. parapertussis*, respectively, as probable.[5,6] Nevertheless, these sequences are not absolutely specific to these species.[6] The sequence IS*481* can be found along with *B. pertussis* in *B. holmesii* and in some strains of *B. bronchiseptica*.[6]

Table 1
Algorithm of multimarker RT-PCR assays for the identification of the main species of *Bordetella* spp.

Interpretation	Result of the assays				
	IS*482*	BPTP	BPTD_0837	IS*1001*	hIS*1001*
B. pertussis	+	+	+	–	–
B. parapertussis	–	–	–	+	–
B. holmesii	+	–	–	–	+
B. bronchiseptica	±	+	–	±	–

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