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Case Report

Non-toxigenic *Corynebacterium diphtheriae* in hallux ulceration

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Abstract

Introduction: Toxigenic *Corynebacterium diphtheriae* causes classical diphtheria. Skin infections by toxigenic or non-toxigenic *Corynebacterium diphtheriae* are prevalent in the tropics but are rarely reported.

Case presentation: We report the identification of a non-toxigenic *Corynebacterium diphtheriae* (biovar Gravis) isolate in a 52-year-old Cambodian male. The patient presented purulent and non-healing ulcerations on the right hallux. The wound has healed after 7 days of antibiotic therapy with a favourable outcome.

Conclusions: This case represents, to our knowledge, the first report of *Corynebacterium diphtheriae* in Cambodia in the last 10 years, and highlights the lack of diagnosis and notifications of diphtheria. It is important to raise awareness among clinicians and to set up diphtheria surveillance in Cambodia.

Key words: Cambodia; *Corynebacterium diphtheriae*; hallux ulceration.

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Introduction

Corynebacterium diphtheriae (C. diphtheriae) was described in 1884 by Loeffler as the causative agent of diphtheria [1]. Toxigenic *C. diphtheriae* and non-toxigenic *C. diphtheriae* (NTCD), are also responsible for skin infections with a chronic, non-healing ulcerative clinical entity, prevalent in endemic areas of the tropics. Even though the diphtheria vaccine elicits the production of antibodies directed against the diphtheria toxin as well as various bacterial components, *C. diphtheriae* infections, caused by toxigenic and non-toxigenic strains, may re-emerge given the low vaccination coverage in some settings [2–5]. Here, we report the identification of a non-toxigenic *C. diphtheriae* isolate in a hallux ulceration of a Cambodian patient.

Case Report

A 52-year-old Cambodian male, with no travel history, living in Prey Veng province with a history of type 2 diabetes and hypertension consulted his pharmacist for ulceration on the right hallux, which had been present for 5 days. The lesion was purulent, painful, and reddened but the patient did not present with a fever. The pharmacist empirically dispensed to the patient 250 mg ampicillin four times per day and 250 mg cloxacillin four times per day without establishing a diagnosis. After 3 days of treatment without apparent clinical response, the patient consulted his general practitioner at Mercy Medical Center Cambodia in Phnom Penh. Apart from the ulceration, the physical examination was unremarkable. A swab of the necrotic lesion was sent to the Medical Biology Laboratory of the Institut Pasteur du Cambodge (IPC) for bacteriological analysis. Polymicrobial growth was observed after 24 hours at 35 °C on both chocolate agar and selective sheep blood agar with nalidixic acid and colistin. Identification of different bacterial colonies initially performed by MALDI-Biotyper (Bruker Daltonics, Bremen,

Germany) revealed the presence of *C. diphtheriae* along with methicillin-susceptible *Staphylococcus aureus* and high-level penicillinase-producing *Escherichia coli*.

The isolate was sent to the National Reference Center laboratory (France) for further characterization and confirmation of both identification and antibiotic susceptibility. The identification of *C. diphtheriae* was confirmed using both a real-time multiplex Polymerase chain reaction (PCR) assay [6] and a biochemical test (API Coryne, bioMérieux, France). The latter test also allowed determining that the biovar was of type Gravis. The multiplex PCR assay also tests for the diphtheria toxin gene (*tox*) presence and revealed that the isolate was non-toxigenic. These assays do not allow differentiating between *C. diphtheriae sensu stricto* and two novel species recently described, *C. belfantii* and *C. rouxii* [7,8]. Genomic sequencing was therefore performed. It showed that the isolate belonged to *C. diphtheriae sensu stricto*, and also confirmed that the isolate did not carry the *tox* gene. Further, the genomic sequence showed that the isolate (FRC1098) did not carry any resistance gene. Genotyping by the multilocus sequence typing (MLST) approach showed that the isolate was of MLST genotype ST779 [9].

Antibiotic susceptibility testing of the *C. diphtheriae* isolate was performed at IPC with the disk diffusion method according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) 2019 guidelines [10] and revealed resistance to penicillin (zone of inhibition diameter, 17 mm). The isolate was susceptible to erythromycin, clindamycin, vancomycin, ciprofloxacin, gentamicin, linezolid, tetracycline, co-trimoxazole, and rifampicin. Resistance to beta-lactam antibiotics was confirmed based on Etest (bioMérieux, France), which allowed determining the following minimal inhibitory concentrations (MIC): penicillin (0.25 mg/L), amoxicillin (0.25 mg/L), ceftriaxone (1 mg/L) and ceftazidime (8 mg/L).

Subsequent investigations regarding the patient's immunization status revealed he had never been vaccinated against diphtheria. The patient received 7 days of antibiotic therapy composed of co-trimoxazole twice a day and clindamycin 300 mg three times per day, a regimen covering all the bacterial species found in the culture. During a follow-up visit 8 weeks after ceasing antibiotic therapy, the wound had healed and the patient remained in good condition.

Discussion

We report on a cutaneous clinical case in a 52-year-old patient with a *tox*-negative *C. diphtheriae* of biovar Gravis and genotype ST779. Real-time PCR and genomic sequencing were used to confirm the initial identification by MALDI-TOF, as the latter does not differentiate *C. belfantii* and *C. rouxii* due to the lack of reference spectra in current commercial databases. The strain is considered resistant to penicillin according to breakpoints of EUCAST 2019 recommendations for both the disk diffusion and Etest methods [10]. However, the interpretation of the diameters and MIC values must be cautious, as the breakpoints for corynebacteria were developed for species other than *C. diphtheriae*. In addition, the genomic sequencing of the isolate showed the absence of antibiotic resistance genes and a recent study on the natural distribution of MICs in *C. diphtheriae* revealed that the strain may be considered susceptible [11]. Our investigations further showed the MIC of ceftriaxone to be higher than that of penicillin, which agrees with this same study. As in most cases when isolating a non-toxigenic *C. diphtheriae* strain from a skin wound, the bacterial culture was polymicrobial, including in this case both *Staphylococcus aureus* and *Escherichia coli*. The polymicrobial character of the bacterial culture and the absence of secretion of diphtheria toxin highlight the difficulty of attributing the infection to the *C. diphtheriae* isolate, as it could act as a co-pathogen or colonizer of a pre-existing wound caused by another pathogen. In addition, the first treatment dispensed by the pharmacist based on antibiotics of the penicillin family should have been effective on the *C. diphtheria* isolate, which does not carry any resistance gene. However, this antibiotic therapy was expected to be ineffective on the two other isolated species. There is currently no consensus on the definition of cutaneous diphtheria, which has been defined as a chronic ulcer growing *C. diphtheriae* from a wound specimen regardless of toxigenic character [12], whereas others consider that cutaneous diphtheria is caused specifically by toxigenic strains [13].

Currently, the Cambodian national immunization program provides diphtheria vaccination free of charge at health centers to children at 6, 10, and 14 weeks of age [14]. However, the 52-year-old patient of this report did not receive any diphtheria vaccine during his childhood, nor any boosters. The national expanded program on immunization (EPI) was launched officially in October 1986 in Cambodia [15].

Although diphtheria was endemic in Cambodia in the 1980s, the latest report dates from 2010, with rare cases reported to the World Health Organization (WHO) between 2000 and 2010 [16]. However, several diphtheria cases have been described during the last decade in South-East Asia countries such as Thailand,

Laos, Myanmar, Vietnam, Malaysia, and Indonesia, including a similar case of foot infection with a non-toxigenic *C. diphtheriae* biovar Gravis in a 16-year-old girl, who had traveled to Thailand [17–23]. The present finding of *C. diphtheriae* in Cambodia highlights the fact that this pathogen should be considered for microbiological analyses in a country where the laboratory capacities are limited and where antibiotics are freely dispensed by pharmacies without bacteriological documentation and medical prescription. In the absence of laboratory capacity and appropriate identification and reporting of the cases, the burden of the disease is likely to be severely underestimated. It would be highly beneficial to improve laboratory capacity with the possibility to detect the *tox* gene, and ideally to test for the production of the diphtheria toxin locally. PCR alone cannot provide a definitive result about toxigenicity, as some *C. diphtheria*e isolates possess the *tox* gene, but do not express the toxin. Besides, PCR technology is not available in many Cambodian laboratories. Therefore, the implementation of the phenotypic Elek's test for defining toxin production would be advantageous, even though it is longer to obtain and less sensitive than PCR [24, 25]. In the present investigation, 6 weeks have elapsed between the initial strain identification in Cambodia and the molecular screening of the toxin in France, which is not useful for patient care in the event of a toxigenic strain. Rapid and reliable diagnosis is also critical to screen patients and their close contacts for *tox*-positive *C. diphtheriae* pharyngeal carriage. There is currently no national surveillance program for diphtheria in Cambodia, and this case report highlights the importance of setting up such a system, which would allow for prompt and effective patient care and could guide prevention and control actions. Finally, even though vaccine coverage for diphtheria in Cambodia is estimated at more than 90% as recommended by the WHO, only primary diphtheria immunization is performed and no booster dose is administered [14, 26]. In this context, a Cambodian seroprevalence study would be useful to evaluate the level of protection of the Cambodian population, given the high proportion of suboptimal protection even in the population of countries that provide several boosters [27].

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