Rapid communications

Imported toxigenic cutaneous diphtheria in a young male returning from Mozambique to Norway, March 2014

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In March 2014 a 20-year-old man was diagnosed with cutaneous diphtheria at St. Olavs University Hospital in Trondheim, Norway on his return from Africa. The man had been in Mozambique since autumn 2013 and had experienced persistent skin ulcer infections. His was in good general health. Toxin-producing *Corynebacterium diphtheriae* was grown from a wound specimen. He had completed the national childhood vaccination programme and received a diphtheria vaccine booster dose in 2005. Screening of close contacts revealed an asymptomatic person colonised with non-toxigenic *C. diphtheriae*.

Case report and laboratory diagnosis

On 23 March 2014, one week after his arrival from Mozambique to Norway, a 20-year-old man presented at the Municipal Emergency Department in Trondheim with a history of skin ulcer, located on the right big toe that had lasted since approximately five to six weeks. He had been working in an orphanage in Mozambique with three other schoolmates from Norway since autumn in the previous year. The patient recalled having had similar leg ulcers lasting for several weeks from October 2013, acquired after his arrival at the orphanage. He could remember some insect bites, as well as minor trauma after he had played football in open toe sandals during his stay there. These ulcers healed after he had received amoxicillin/clavulanic-acid orally for one week, prescribed by a local physician in Mozambique.

At the Emergency Department in Trondheim, the examining physician suspected an infection caused by pyogenic bacteria and a wound specimen was requested for aerobic culture and screening for meticillin-resistant *Staphylococcus aureus* (MRSA). A treatment consisting of oral dicloxacillin tablets 500 mg four times daily was initiated.

After 24 hours of incubation on blood agar and chocolate agar, abundant growth of almost pure culture of small, 1–2 mm in diameter, white, non-haemolytic colonies mimicking normal bacterial skin flora, was observed. A wet mount demonstrated short rods. Using matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS) on a Microflex LT mass spectrometer (Bruker Daltonics) with BioTyper 3.2 software database, the isolate was identified as *Corynebacterium diphtheriae*. Score values of 2.113 and 2.041 were interpreted as reliable species identification, as recommended by the manufacturer.

On Tinsdale selective medium (Tinsdale agar base: Difco product nr.278610 and Tinsdale enrichment Difco product nr.234210, BD Diagnostics – Diagnostic Systems), the isolate displayed characteristic deep brown colonies with halos after 24 hours of incubation.

Laboratory investigation at the National Reference Laboratory

On 26 March the isolate was sent on Amies transport medium to the National Reference Laboratory for Diphtheria at the Norwegian Institute of Public Health (NIPH), Oslo, and diphtheria toxin *tox* gene was detected by polymerase chain reaction (PCR) [1] on 28 March. Diphtheria toxin production was analysed by modified Elek test [2] and reported positive on 29 March. The strain was identified as *C. diphtheriae* biotype mitis by API Coryne v3 system (BioMérieux, France, code: 1010364) and supplementary tests (nitrate reduction

positive, glycogen fermentation positive, not lipophilic and forming large colonies (>1 mm in diameter after 24 hours of incubation)).

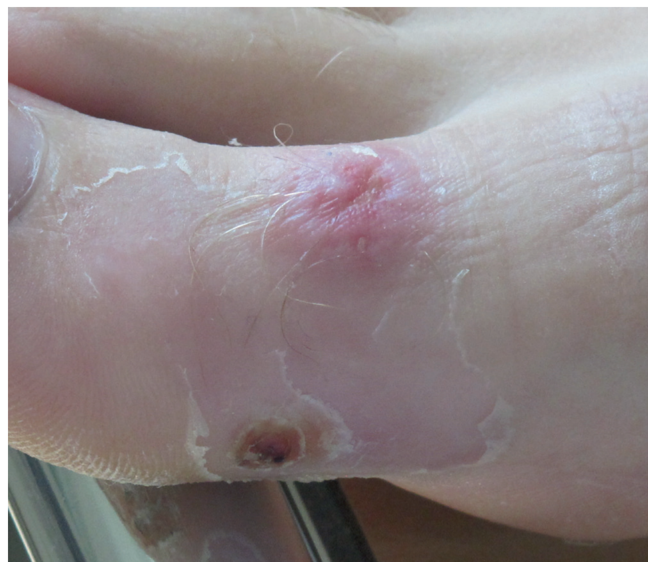
Minimum Inhibitory Concentration (MIC) for benzylpenicillin was 0.125 mg/L determined by Epsilometer (E) test on a blood agar plate. The European Committee on Antimicrobial Susceptibility Testing (EUCAST) have no species specific breakpoints for *C. diphtheriae*, but the strain can be categorised as susceptible to benzylpenicillin according the EUCAST recommendations for non-species related clinical breakpoints [3].

Patient follow-up

After diagnosing *C. diphtheriae* infection on 25 March, and prior to obtaining the results of antimicrobial susceptibility testing, the treatment was changed from dicloxacillin to oral phenoxymethylpenicillin tablets 660 mg four times daily, according to the suggested regimens from The Sandford Guide To Antimicrobial Therapy 2014 [4]. The patient vaccination history was reviewed; he had completed the national childhood vaccination programme and received a diphtheria vaccine booster dose in 2005. The next day, on 26 March, the patient was admitted to Department of Infectious Diseases, St. Olavs Hospital, Trondheim, where he was isolated. He was afebrile and in good general health. There were no signs of pharyngeal involvement, C-reactive protein (CRP) was <5 mg/L (norm: 0–5 mg/L) and the leukocyte count was 9.1×10^9/L (norm: $3{,}7–10 \times 10^9$/L). An ulcerative, non-inflammatory wound was observed on his right big toe (Figure). He received intravenous benzylpenicillin treatment 1 million IU x 4, during the 24 hours hospitalisation and oral phenoxymethylpenicillin 660 mg two tablets three times daily for two weeks after discharge.

Figure

Ulcerative, non-inflammatory wound on the right big toe of a patient with cutaneous diphtheria, Norway, March 2014



Control measures and contact tracing

Immediately after diagnosing *C. diphtheriae* infection on 25 March, the case was reported by phone to the local Medical Officer in the municipality and NIPH in accordance with the Communicable Disease Act. In collaboration with the treating physician and the local Community Medical Officer, contact isolation precautions were implemented until the patient was admitted to the hospital. After discharge, he was isolated in his home until two control cultures (throat, nasal and wound swab) taken on 9 April and on 10 April and cultivated on Tinsdale selective medium, were negative on 15 April.

Tracing of close contacts was initiated on 25 March and oral erythromycin capsules 500 mg two times daily for seven days were given prophylactically. A booster diphtheria vaccine dose was offered to all contacts who received the last diphtheria vaccine dose more than five years prior and complete vaccination to those who had not been vaccinated in the primary childhood programme. Observation of close contacts in their homes (for fever, throat pain) in the following seven days after they had been exposed to the index patient was recommended. Throat and nasal specimens were also collected from these close contacts, which included 11 close family members, four friends and the primary examining physician. The specimens were cultivated, as well as patient control samples, on Tinsdale selective medium at St. Olavs Hospital, Trondheim; all were found negative for *C. diphtheriae*.

The index patient was attending the boarding school in Hurdal (located 70 km north of Oslo) and on his arrival to Norway, before he visited his family, the boarding school was the accommodation where he spent the first week of his vacation. During this week, the index patient and other schoolmates had eaten together in the kitchen of the boarding school and some of them had shared the bathrooms and sleeping rooms. Three other schoolmates, who had been working in Mozambique at the same time as the index patient, had also arrived to the boarding school. Taking this into consideration and according information obtained by local Medical Community Officer in Hurdal, throat and nasal specimens from 53 close contacts: 28 schoolmates and 25 other contacts – employees in the school and close contacts out of the boarding school –, were collected and sent to Akershus University Hospital in Oslo. All samples were cultivated on Tinsdale selective agar media (Tinsdale agar base, Oxoid product nr.CM 0487) and were found negative, except one throat swab from one of his schoolmates, a travel companion in Mozambique. This isolate was sent to NIPH and identified as *C. diphtheriae* biotype mitis by API Coryne v3 system (BioMérieux, France, code: 1010324) and supplementary tests. The isolate had the same biotype as the isolate from the index case, but differed by being lactose positive and toxin negative (both with *tox* gene PCR and modified Elek test).

This schoolmate, who had been working in Mozambique at the same orphanage as the index patient, may also have been infected during his stay there with another strain of *C. diphtheriae*, the non-toxigenic one, but was asymptomatic and discovered by screening. Molecular diagnostic that is planned in the near future, the genome sequencing of both isolates, should reveal if it was the identical strain infected by lysogenic *tox* phage in the index patient or two different strains.

After diagnosing *C. diphtheriae* throat colonisation, the vaccination history of the contact patient was reviewed; he had received a booster diphtheria vaccine dose in 2012 and was not offered a new one. He received the oral erythromycin capsules 500 mg two times daily for seven days and was isolated in the boarding school (separate room and bathroom), until two control throat specimens sampled at different times were negative. The vaccination history of the other 52 persons included in screening in Hurdal was reviewed and 29 close contacts who had been vaccinated more than five years prior received a booster diphtheria vaccine dose. All close contacts were given the oral erythromycin capsules 500 mg two times daily for seven days, with some exceptions (one pregnant woman and one child).

One of the schoolmates, who had also been working in Mozambique at the same time as others, had noticed skin ulcer on his leg, but in his case, the ulcer healed spontaneously and at the time of screening, the wound swab taken from the scar area was negative for *C. diphtheriae*.

Background and epidemiological situation

Diphtheria is caused by toxigenic strains of *C. diphtheriae*, *C. ulcerans* and *C. pseudotuberculosis*. It can result in an acute bacterial toxic infection of the upper respiratory tract or in cutaneous infection, which is generally a milder variant of the disease.

Cutaneous diphtheria is usually described as a chronic ulcer, often following insect bites or minor trauma. The incubation period is on average two to four days for respiratory tract diphtheria but is not so well defined for cutaneous infection. Immunised persons seldom develop systemic toxic manifestations and the slow absorption of toxin from skin lesions induces production of high antibody levels [5]. Isolation of *C. diphtheriae* by culture may be difficult due to normal throat or skin flora and other pathogens present; therefore selective media should be employed when diphtheria is suspected.

In reports from several European countries [6-9], isolates causing cutaneous diphtheria were mainly imported and toxigenic. Epidemiological control and vaccination are important measures in reducing the possibility of establishment of a reservoir for secondary transmission of both cutaneous and respiratory diphtheria [10].

Diphtheria is rarely diagnosed in Norway due to high vaccine coverage. During the period from 1975 to 2013 only five cases of throat diphtheria or colonisation have been reported to the Norwegian Surveillance System for Communicable diseases (MSIS). In 1992, a young man from the county of Finnmark was infected after contact with a person from Russia. In 2008, a mother and her child were diagnosed with throat diphtheria after visiting Latvia. After their return to Norway, the father and one other child were infected [11,12].

A brief report of the present case has been covered in the bulletins of NIPH on 2 April 2014 as the first diagnosed toxigenic cutaneous diphtheria in Norway [13].

Discussion

Cutaneous diphtheria is endemic in some eastern European countries (Latvia, Russia) and many parts of the world (Brazil, Eastern Mediterranean region, Haiti, the Indian subcontinent, Indonesia, Nigeria and Philippines) [5] and physicians should be aware of the possibility of diphtheria in patients returning from visits/travel in endemic areas. *C. diphtheriae* can survive up to three months in floor dust [14] and in endemic areas with tropical climate this can be a likely source of infection/transmission.

The report illustrates the importance of diagnosing diphtheria cases as soon as possible, given the amount of resources needed for subsequent contact-tracing and control measures, which is likely to increase when detection of an initial case is delayed.

The diagnosis of diphtheria in the present case emphasises the importance of detailed clinical and epidemiological information given by the examining physician as well as access to modern diagnostic modalities. MALDI-TOF MS is easy to use, cost effective and enables rapid species identification in a couple of minutes [15]. The usefulness of MALDI-TOF MS as a tool for reliable *C. diphtheriae* identification was recently investigated by Konrad et al. [16]. They correctly identified to the species level all 90 potentially toxigenic *Corynebacterium* strains and proposed an algorithm for fast and reliable identification of *C. diphtheriae* incorporating MALDI-TOF MS, real-time *tox* PCR and Elek testing. This workflow was shown to be both rapid and effective in our case.

The participation of laboratory in Trondheim in the United Kingdom National External Quality Assessment Service for Microbiology (UK NEQAS) also proved beneficial. One isolate of toxin-negative *C. diphtheriae* was recently distributed by UK NEQAS on 3 February 2014 (distribution nr.3361), and was successfully identified by MALDI TOF MS.

The collaboration of the laboratory in Trondheim with NIPH, performing the *tox* gene PCR, Elek testing, biotyping and susceptibility testing, and with the local Community Medical Officer, proved very efficient and

optimal in the present case, when encountering a rare and potentially severe infectious disease. The contact patient diagnosed with non-toxigenic *C. diphtheriae* remained asymptomatic. Non-toxigenic strains of *C. diphtheriae* are recently recognised as emerging pathogens across Europe [17]. Such strains, however, can convert to toxigenicity by infection with lysogenic tox phage [18,19]. The circulation of resident non-toxigenic strains in the community thus can represent an ongoing risk by conversion to highly virulent strains following lysogenisation.

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Conflict of interest

None declared.

Authors' contributions

Wrote the manuscript: AJ, MS, ATM, PBS, ES, TS, KR, HB, KB; performed clinical investigations: PBS, TS; performed laboratory investigations: AJ, MS, ATM, HB, KB; performed epidemiological investigations: ES, KR, HB.

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