

Colonisation with toxigenic *Corynebacterium diphtheriae* in a Scottish burns patient, June 2015

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On 12 June 2015, *Corynebacterium diphtheriae* was identified in a skin swab from a burns patient in Scotland. The isolate was confirmed to be genotypically and phenotypically toxigenic. Multilocus sequence typing of three patient isolates yielded sequence type ST 125. The patient was clinically well. We summarise findings of this case, and results of close contact identification and screening: 12 family and close contacts and 32 hospital staff have been found negative for *C. diphtheriae*.

Case report

On 9 June 2015, a 20 year old female patient had routine swabs taken from the discharge of recent wounds, following reconstructive surgery. The samples subsequently grew *Corynebacterium diphtheriae*.

The patient had contracted severe 30% burns to her face, neck, chest, arms, and thighs as a child. She was followed up regularly by the plastic surgery team for management of burn contractures and skin grafts, and had no other relevant past medical history. On examination her wounds were not erythematous or ulcerated, and appearances were consistent with satisfactory healing.

The patient's childhood vaccination history was fully up to date as per the UK (UK) childhood vaccination schedule recommended [1], and included vaccination for *C. diphtheriae* infection.

Further questioning revealed no history of foreign travel at all in preceding years, or of close contacts who had travelled abroad, and there was no known animal contact. The patient had no clinical features of cardiovascular, neurological or cutaneous manifestations of *C. diphtheriae*. She reported a sore throat which was unremarkable on clinical examination with no membrane present. Following confirmation on 12 June of *C.*

diphtheriae in swabs taken on 9 June, the patient was commenced on oral erythromycin 500mg, six hourly as per Public Health England (PHE) guidelines for the public health control and management of diphtheria. The treatment only started on 16 June, after some delays. The treatment continued for 14 days [2] and booster vaccinations were arranged. As the patient was very well, antitoxin was not administered.

Throat swabs and repeat skin swabs taken on 16 June, again grew toxigenic *C. diphtheriae*. Results were available on 19th June and were identified by using MALDI-TOF and Hoyle's Tellurite agar.

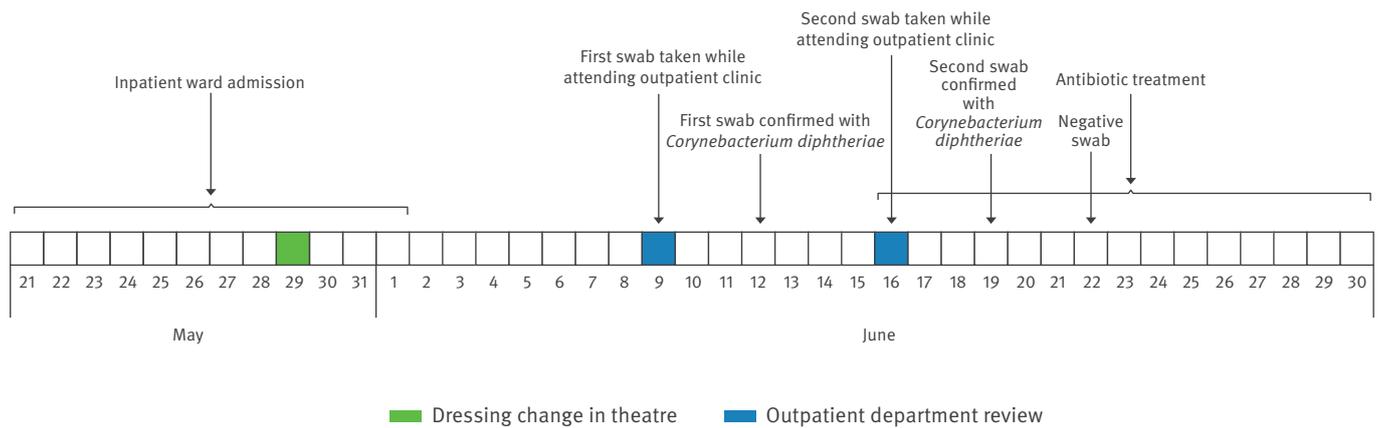
Nose, wound and throat swabs were taken on 22 and 23 June. The results of the swabs were available on 26 June and were all negative for *C. diphtheriae*.

Laboratory findings

The organism was initially identified on the 12 June, by matrix-associated laser desorption ionisation time-of-flight (MALDI-TOF) machine used for automated identification of microorganisms (Bruker); with a MALDI score of 99.9%. This test was repeated and a Gram-stain demonstrated Gram-positive bacilli. Hoyle's Tellurite agar and the API Coryne system (bioMérieux) were subsequently used to confirm the identity of the strain as *C. diphtheriae*. The isolate was then sent to the PHE diphtheria national reference laboratory in Colindale, where it was confirmed as PCR- positive for the *toxA* gene, and phenotypically positive for toxin production by the Elek immunodiffusion test. The two later isolates from the throat and wound swabs were also confirmed as *C. diphtheriae*, *tox-A* -positive and Elek-positive. The genotypic relationship of all three *C. diphtheriae* isolates was characterised using the multilocus sequence typing (MLST) scheme described by Bolt et al. [3] comprising the seven *C. diphtheriae* housekeeping genes *atpA*, *dnaE*, *dnaK*, *fusA*, *leuA*, *odhA*, and *rpoB*. Allelic

FIGURE

Timeline for colonisation with toxigenic *Corynebacterium diphtheriae* in a Scottish burns patient, June 2015



profiles and sequence type (ST) designations for each strain were obtained via the PubMLST database curated by the Pasteur Institut, Paris, France (<http://pubmlst.org/cdiphtheriae/>). All three isolates had the allelic profile 19,4,8,1,3,3,13 corresponding to ST 125.

Antibiotic susceptibility testing was undertaken using Clinical and Laboratory Standards Institute breakpoints [4] and the isolate tested sensitive to erythromycin, ciprofloxacin, meropenem, vancomycin, clindamycin, and clarithromycin. It was of intermediate sensitivity to penicillin. EUCAST breakpoints were not used due to lack of definitive breakpoints for *C. diphtheriae*.

Contact tracing

A timeline was prepared by our infection control team in conjunction with the clinical and public health teams, using the maximal incubation period of 10 days as a guide (Figure), with the caveat that the patient reported increased discharge from the wound before 31 May. The patient had spent most of this time as an outpatient at home, with the exception of being an inpatient for one day at the start of the lookback period, and presented to our clinic on 9 June 2015. Contact tracing was undertaken on all 12 family and other close contacts; this included friends, partner and relatives. Additionally, 32 hospital staff were identified as having had a relevant exposure during the patient's inpatient stay, such as being involved with changing wound dressings. Nose and throat swabs were taken from all of them, and Hoyle's Tellurite agar and MALDI-TOF techniques were used to screen specifically for the organism.

The vaccination histories of the patient's two year old child, family contacts as well as all hospital staff contacts, were checked and found to be complete as per UK recommendations. All identified family and close contacts and hospital staff were commenced on chemoprophylaxis with erythromycin at British National Formulary recommended doses, and excluded from work and encouraged to self-isolate until swab

results were confirmed as negative. All contacts tested negative.

A memorandum was sent to all medical and nursing staff to enquire about recent foreign travel to exclude a hospital acquired infection.

The source of the organism remains unidentified.

Discussion

C. diphtheriae is an aerobic Gram-positive bacillus with a worldwide distribution. It causes diphtheria, a potentially life-threatening upper respiratory tract infection with a mortality rate of 5–10% in untreated cases [2,4,5]. As it is a vaccine preventable illness, it is now very rare in the European Union. Classic diphtheria is caused by bacteriophage-associated exotoxin-producing strains resulting in necrosis of infected cells and a classic fever, sore throat and tonsillar membrane. Non-toxicogenic strains usually cause a milder illness or asymptomatic colonisation. Other manifestations of disease include cardiac syndromes (such as myocarditis), cutaneous lesions as well as neurological syndromes [5-7].

Humans are the main reservoir and transmit infection via respiratory droplets as well as direct contact. Respiratory and contact precautions are therefore required in an inpatient setting. The incubation period ranges from 1 to 10 days, and untreated individuals may be infectious for 2 to 4 weeks [2,5]. Treatment includes intravenous or oral macrolide therapy, intramuscular penicillin and antitoxin in severe illness.

Toxicogenic *C. diphtheriae* is relatively rare in the UK and United States but prevalence is high in the Indian subcontinent and other parts of Asia, Eastern Europe, Africa, South and Central America. Numbers of toxicogenic *C. diphtheriae* and *C. ulcerans* cases are one to six per year (2008–2014) in England [8]. Around 35 to 75 isolates of *C. diphtheriae* are submitted to the national reference laboratory per year (2010–2014)

and the majority of these are subsequently shown to be non-toxigenic [9,10].

Of 272 *C. diphtheriae* strains confirmed by the national reference laboratory from 2010 to 2014, 69% were *C. diphtheriae* biovar *gravis*, 26% biovar *mitis*, and 4% biovar *belfanti* [11]. The strains from this case were all biovar *mitis*. The main risk factor for infection with toxigenic *C. diphtheriae* infection in the UK is travel to an endemic area or contact with others returning from such an area. For toxigenic *C. ulcerans* infection, the main risk factor is contact with animals including companion animals [9,10]. Neither the case nor the contacts had travelled abroad or had any animal contact.

This case demonstrates the importance of team work, and required close coordination and communication between clinical, infection prevention and control, public health, community health and reference laboratory teams. The organism was first identified by the microbiology team in a clinical laboratory who then contacted the general practitioner, clinical, infection control and public health teams. The ensuing multidisciplinary investigation required parallel contact tracing within and outwith the hospital settings, clinical assessments of the case and contacts, infection control investigations within the hospital, as well as close liaison with GPs of the case and contacts to guide chemoprophylaxis regimes as well as exclusion from work. Lines of communication between different multidisciplinary team members included phonecalls, emails as well as a teleconference and this was crucial in ensuring a coordinated and thorough investigation. Nevertheless, the case raised some questions; for example, whether responsibility for liaison with the general practitioner as well as the patient directly lay with the microbiology team or local health protection unit. Additionally, would a Standard Operating Procedure or protocol be warranted to guide teams when such situations arise?

Given the relative rarity of toxigenic diphtheria, it may be best to handle each case on an individual basis. With the advent of new molecular or rapid diagnostic technologies, such as the MALDI-TOF, it is possible that we may detect a greater number of any pathogen (not restricted only to *C. diphtheriae*) which hitherto may have gone unidentified, and this may have both clinical and epidemiological implications. Indeed, if a Gram stain had been done in the first place (which would traditionally have been the case), it might have just been discarded as a diphtheroid contaminant.

Due to the highly contagious nature of *C. diphtheriae* and its relative rarity in Scotland, where only two cases were reported between 2000 and 2013 [12], this case is of particular interest, especially as the strain involved was a toxin producing strain. The ST of the three isolates from this patient (ST 125) was first designated for an isolate of *C. diphtheriae* from 2009 from France. As of 24 November 2015, no other isolates with ST 125 have been diagnosed and the

two closest profiles have four of seven alleles in common; ST 232 (19,1,20,1,18,3,13) isolated in 2011 from Polynesia and ST 261 (13,4,8,44,3,23,13) isolated in 2009 from a patient in England [8], from a patient with a chronic leg ulcer from a prosthetic knee infection. Interestingly, this latter patient had themselves lived on a Polynesian island. The source of the organism for the case described here remains unclear, and little is known of the true epidemiology in Scotland.

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Conflict of interest

None declared.

Authors' contributions

Dr Ashutosh Deshpande: first main author, wrote first draft and co-ordinated subsequent redrafts;

Dr Teresa Inkster: second main author, wrote first draft, helped with all redrafts, provided infection prevention control information; Ms Kate Hamilton: prepared timeline figure, provided information regarding hospital contact tracing and other aspects of infection prevention and control;

Dr David Litt: supported redraft of article and performed MLST analysis; Dr Norman Fry: supported redraft of article and molecular testing of isolates; Dr Iain T R Kennedy: redrafted the article, provided epidemiological information including tracing of personal contacts and vaccination history, helped write discussion and management of case; Mrs Jacqueline Shookhye-Dickson: redrafted the article, provided epidemiological information including tracing of personal contacts and vaccination history, helped write discussion and management of case; Dr Robert L R Hill: helped to redraft the article and provide reference laboratory input.

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