

2 VACCINE-PREVENTABLE DISEASES

a Diphtheria

Update on diphtheria outbreak, Kwa-Zulu Natal

The July Communiqué reported the latest suspected case of diphtheria involving a 12-year-old HIV-positive boy, well controlled on antiretroviral therapy from a town in Ugu District, KwaZulu-Natal Province (KZN). The case has value in proving the importance of laboratory processes in outbreak investigations. The boy presented to the casualty department of the local hospital on 24 July 2015. He had a sore throat and 'bull-neck' for two days, and subsequently developed shortness of breath and difficulty in swallowing – features in keeping with respiratory diphtheria. His vaccination status was unknown. On examination, he was in respiratory distress with an oxygen saturation of 80% in room air. His uvula was inflamed and a grey-white tonsillar exudate was noted. He was intubated but unfortunately had a cardio-respiratory arrest while at the X-ray department and demised. Two throat swabs and one nasal swab taken before death were submitted to the laboratory of the district hospital, but 24 hours later, no bacterial growth was observed.

According to the NICD guidelines, the case was classified as a 'possible case of diphtheria', as although there was no epidemiological link with a confirmed case, there had been cases in Ugu District, and it was not wise to discard the case in the context of an outbreak. Fortunately the family agreed to a post-mortem that revealed a clinical picture of extensive upper and lower respiratory tract disease secondary to infection with *Streptococcus pyogenes*, which grew profusely from all upper airway specimens. Extensive oedema, enlarged lymph nodes and pus was observed in and around tonsils, uvula and epiglottis, but no pseudomembrane was present. Bilateral pneumonic consolidation was present (more on the right lung) associated with a right-sided pyothorax. Pleural and pericardial samples were also sent which did not yield any organisms of clinical significance. In addition, extensive ulcerative and healed skin lesions resembling impetigo were observed and a swab from the leg also yielded growth of *S. pyogenes*. All the samples taken from the post-mortem were culture negative for *C. diphtheriae* on culture and PCR.

In the light of these findings, this case was removed from the diphtheria outbreak line list. This means that the outbreak is currently under control. No further cases of diphtheria have been identified

since 12th June 2015.

Diphtheria: molecular epidemiology of *Corynebacterium diphtheriae* outbreak isolates

The Centre for Respiratory Diseases and Meningitis (CRDM) at the NICD received 21 *C. diphtheriae* isolates collected from suspected cases and contacts during the KZN diphtheria outbreak (March – June 2015). *C. diphtheriae* identification of positive cultures was confirmed by matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) technology. Toxin production and the presence/absence of the A and B subunits of the *C. diphtheriae* toxin (*tox*) gene were confirmed by the Elek test and real-time PCR, respectively.^{1,2} Whole genome sequencing was conducted on all 21 isolates together with three toxin-positive *C. diphtheriae* controls (including PW8, the vaccine-type strain isolated in the 1890s), a toxin-negative *C. diphtheriae* control, two historical *C. diphtheriae* clinical isolates from South Africa circa 1980s (one toxin producing and the other non-toxigenic), and *C. ulcerans*, *C. bovis* and *C. stratum* control organisms as outliers. Multilocus sequence typing (MLST), a sequence-based typing method that combines alleles of seven housekeeping genes to form a sequence type (ST), was used to characterise the isolates.³ STs were extracted from whole genome data and compared to all available STs (n=409) listed in the global MLST database (<http://pubmlst.org/cdiphtheriae/>). Genomes of KZN outbreak-associated isolates were compared with control and historical isolate genomes to determine genetic relatedness.

Based on the clinical definition of respiratory diphtheria and results of laboratory testing, eleven isolates from case-patients were toxin producing. An additional six toxin-producing isolates were from asymptomatic carriers epidemiologically linked to cases. Toxin production was confirmed in all isolates that were genotypically positive for the *tox* gene. Our laboratory received two isolates from one case-patient: a toxin-producing isolate and a non-toxigenic isolate. Three additional non-toxigenic *C. diphtheriae* isolates were submitted. These had been isolated from two cases with suspected respiratory diphtheria and one case with suspected cutaneous diphtheria.

Two novel sequence types were identified among the outbreak isolates, none of which were related to

any other sequence types listed in the global database. All 17 toxin-producing isolates from the KZN outbreak (cases and contacts) had the same sequence type (ST-378) and clustered together on the whole genome phylogenetic tree. A second cluster comprised the four non-toxigenic KZN isolates (including the cutaneous *C. diphtheriae*) and one of the historical non-toxigenic clinical isolates from 1980 – all five isolates were of the same sequence type (ST-395). The toxin-producing historical isolate clustered separately and had a different sequence type that was also novel (ST-402). The ATCC and NCTC *C. diphtheriae* control isolates had different sequence types and clustered individually.

Two unusual features were noted during the laboratory investigations. Firstly, two unrelated isolates with different genotypes (one toxin-producing and the other non-toxigenic) were collected from the same patient at the same time. To the best of our knowledge laboratory error was excluded but this cannot be ruled out entirely. Secondly, a case infected with a non-toxigenic strain was epidemiologically linked to three asymptomatic individuals who were carriers of a toxin-producing strain with a different genotype to that isolated from the case. The PCR results correlated with the Elek results initially performed at the NHLS Greenpoint Laboratory in Cape Town, Western Cape Province for each of these isolates. Both these observations remain unexplained at this time.

Molecular typing is essential in outbreak investigation to understand patterns of transmission as well as to monitor the evolution and spread of epidemic clones. These molecular findings illustrate that the outbreak in KZN is caused by two strains. It is not possible yet to determine the origin of these outbreak strains as prior to the KZN outbreak, there are no data describing circulating genotypes in South Africa. The NICD therefore requests all laboratories nationally to submit stored or prospectively-identified *Corynebacterium* isolates to CRDM for molecular characterisation. Laboratory guidelines for *C. diphtheriae* isolation are available on the NICD website.

References

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