Infections with *Corynebacterium diphtheriae*: six years' experience at an inner London teaching hospital

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ABSTRACT

Recently, a preponderance of non-toxigenic strains of *Corynebacterium diphtheriae* has been reported, as has the broadening spectrum of disease caused by these strains. This study presents data on 85 isolates of *C. diphtheriae* over a six year period (1998–2003). Eighty were non-toxigenic isolates from patients with sore throat, and five (one toxigenic) were from cutaneous ulcers in travellers returning from endemic areas. When examined in relation to denominator data provided by the Health Protection Agency (HPA) for the whole of England and Wales, 1998–2002, 75% of all notifications of *C. diphtheriae* for England and Wales originated from the laboratories at University College London Hospitals (UCLH). In some years (1999 and 2001) 95–100% of isolates came from UCLH. We believe that national data do not reflect true incidence, as universal screening for these organisms is not routine policy in many laboratories. The results presented suggest the need for increased clinical and laboratory awareness of this important pathogen.

KEY WORDS: Corynebacterium diphtheriae. Corynebacterium infections.

Introduction

As a result of mass immunisation against diphtheria in Western Europe, there has been a steady decline in the number of cases due to toxigenic *Corynebacterium diphtheriae*. In England and Wales, between 2000–2002, there were four notifications of toxigenic diphtheria, and only one reported death due to diphtheria since 1985.[1]

In an attempt to cut laboratory costs, the need for continued screening of throat and wound specimens for *C. diphtheriae* has been questioned. The call for cessation of routine screening is due to the perception that toxigenic isolates are now rare[2] and that there is now significant loss of laboratory skills in the isolation and identification of this organism. Indeed, many laboratories have stopped screening routine throat swabs for *C. diphtheriae*.[3] Even fewer laboratories screen wound swabs for cutaneous diphtheria in travellers returning from countries in which the disease is endemic. However, the standard operating procedures of the Health Protection Agency (HPA) recommend screening for *C. diphtheriae* in all throat swabs and relevant wound swabs.[4]

Diphtheria remains an important threat to health in many parts of the world. Eastern Europe, the former Soviet Union, India, Pakistan and Bangladesh, and many countries in South-East Asia are all areas in which diphtheria is still endemic.[5] With increasing tourism, diphtheria continues to be a potential health hazard in travellers returning from these areas. Toxigenic strains are life threatening and are a potential outbreak threat, as demonstrated by the epidemic of 1990–1998 in the former Soviet Union.[6] Furthermore, cutaneous diphtheria can cause outbreaks of cutaneous and respiratory forms of the disease.[7] In the UK, 17 patients with cutaneous diphtheria due to toxigenic *C. diphtheriae* were reported in 1995–2002. All were travel-related.[7]

However, a preponderance of non-toxigenic strains has been noticed, as has a widening spectrum of disease caused by these strains.[2,3] One of the important manifestations of non-toxigenic strains is the development of severe and sometimes recurrent sore throat. Early diagnosis of *C. diphtheriae* has implications for treatment and public health intervention. In addition non-toxigenic strains reflect a potential pool of the organism in the community, as some strains that carry the toxin gene may not express the toxin.[8]

Here, laboratory and clinical data of *C. diphtheriae* infections over six years (1998–2003) at University College London Hospitals (UCLH) are presented.

Materials and methods

A retrospective analysis of all *C. diphtheriae* isolations in the UCLH laboratory from 1998–2003 was undertaken. The laboratory serves a catchment area of 480,000, and also serves the Hospital for Tropical Diseases, which is a tertiary referral centre for tropical infections and a UK reference centre for travel medicine.

Patient demographic information was obtained from the clinical microbiology computer database. Total number of isolations of both toxigenic and non-toxigenic strains of *C. diphtheriae* from England and Wales as reported by the HPA for this period was used as denominator data.[1]

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Table 1. Isolates of *C. diphtheriae* by subspecies per year.

Year	*var gravis*	*var intermedius*	*var mitis*	*var belfanti*	Total at UCLH (% of all notifications)	Total notifications from England and Wales (HPA)
1998	7	0	6[2]	0	13 (56.5)	23
1999	13	0	9	0	22 (95.7)	23
2000	8	0	9	0	17 (89.5)	19
2001	5	0	8[1]	0	13(100.0)	13
2002	4	0	4	1	9 (42.9)	21
2003	6	0	4[2*]	1	11	awaited
Total (%)	43 (50.6)	0 (0)	40 (47.0)	2(2.4)	85	–

Figures in parentheses [ ]: number of isolates from tropical ulcer. * one strain toxigenic

Table 2. Number of isolates of *C. diphtheriae* by age group for each year.

Age group	1998	1999	2000	2001	2002	2003	Total (%)
<16	0	1	0	0	0	0	1(1.2)
16–25	4	7	9	3	4	7[1*]	34(40.0)
26–35	4[1]	10	6	6[1]	4	1	31(36.4)
36–45	3[1]	2	1	4	1	3[1]	14(16.5)
46–55	1	0	0	0	0	0	1(1.2)
>55	0	1	0	0	0	0	1(1.2)
Age not known	1	1	1	0	0	0	3(3.5)
Total	13	22	17	13	9	11	85

Figures in parentheses [ ]: number of isolates from tropical ulcer. * toxigenic strain

Table 3. Isolates of *C. diphtheriae* by sex of patient.

Gender	1998	1999	2000	2001	2002	2003	Total (%)
Male	10[2]	16	12	11[1]	6	6[2]	61(71.8)
Female	2	5	4	2	3	5	21(24.7)
Unknown	1	1	1	0	0	0	3(3.5)
Total	13	22	17	13	9	11	85

Figures in parentheses [ ]: strains from tropical ulcer

Laboratory methods

Throat swabs from patients with acute symptomatic sore throat were cultured routinely on Staph-Strept selective agar (Oxoid, Basingstoke, UK) and incubated anaerobically for β-haemolytic streptococci and *Arcanobacterium haemolyticum*. Also, aerobic culture on Hoyle's tellurite agar (Oxoid) was undertaken for *C. diphtheriae*. Tropical ulcer swabs were cultured on Hoyle's medium for *C. diphtheriae*, in addition to the usual laboratory media for pathogens such as *Staphylococcus aureus* and *Streptococcus pyogenes*.

After 48 hours' incubation, grey to black colonies on the Hoyle's medium were Gram-stained to confirm the presence of Gram-positive coryneform bacilli. Preliminary testing for *C. diphtheriae* was performed by inoculation of half a suspicious colony onto Tinsdale's medium (Oxoid) for the production of cyteinase (produced by the classically pathogenic corynebacteria). The other half of the colony was subcultured on blood agar to retain purity. Identification of cysteinase-positive (a brown halo around the colony on Tinsdale's medium) colonies was confirmed using the blood agar subculture in an API Coryne kit (bioMerieux, Basingstoke, UK).

Isolates identified as *C. diphtheriae* were sent to the Diphtheria Reference Unit, Respiratory and Systemic Infection Laboratory, Health Protection Agency, Colindale, London, for further speciation and toxin testing.

Results

Eighty-five isolates of *C. diphtheriae* were recovered over the six-year period. Eighty isolates were from throat swabs and

five from tropical ulcers. All throat isolates were non-toxigenic, as were four of the five isolates from cutaneous lesions. Only one isolate from a cutaneous lesion in a traveller returning from a tropical country in which the disease is endemic tested positive for toxin production.

Table 1 outlines the number of isolations by subspecies for each year at UCLH in relation to the total number of notifications received at the HPA from all of England and Wales for the same year. For 1998–2002, 75% of all *C. diphtheriae* notifications for England and Wales originated from the laboratories at UCLH. In some years (1999 and 2001), 95–100% of isolates were reported from UCLH. The majority of strains were of the *gravis* and *mitis* subspecies, with only two strains of the *belfanti* subspecies. No isolates of the *intermedius* subspecies were recovered.

Table 2 lists the isolates by age group for each year. The majority of isolates were recovered from patients between the ages 16–45 (79 isolates). A male:female ratio of almost 3:1 was apparent for throat isolates (Table 3). All five ulcer isolates were recovered from men.

Discussion

The resurgence of diphtheria as a threat to human health was illustrated by the occurrence of the epidemic in the states of the former Soviet Union between 1990 and 1998.[6] This was mainly due to the breakdown of previously established vaccination programmes. During this period, an increase in the recovery of non-toxigenic strains from patients with sore throat also become apparent.

In the UK, Reacher *et al*. documented the emergence of non-toxigenic strains of *C. diphtheriae* as a cause of recurrent pharyngitis of varying severity.[9] A majority of these isolates originated at UCLH and were attributed to the indigenous population, rather than to travel-related isolations. An interesting report by Wilson *et al*.[10] described a high prevalence of similar non-toxigenic biotypes of *C. diphtheriae* in a population of inner London homosexual men, reflecting transmission within a small community sharing similar lifestyle and behavioural patterns.

Notifications of toxigenic and non-toxigenic *C. diphtheriae* as presented in Table 1 clearly demonstrates that the data presented by the HPA from England and Wales notifications are biased and depend on laboratories screening for the organism.

These findings impact on communicable disease control, as sections of the population that are not screened may transmit infection unknowingly.

Selection against toxigenic strains due to vaccination may explain the predominance of non-toxigenic strains circulating in the population.[2] Furthermore, concern has been expressed about such strains as some may bear the toxin gene but fail to express the toxin.

Groman *et al*. described non-toxigenic, toxin gene-bearing strains as long ago as the early 1980s.[8] Recently, these strains have been shown to represent 20–30% of the current isolates of *C. diphtheriae var mitis* from the countries of Eastern Europe.[11] However, what proportion of the strains contained the toxin gene but remained unexpressed is not known.

In their report, de Benoist *et al*. noted that of 17 patients with cutaneous diphtheria in the UK in 1995–2002, 15 patients gave a vaccination history – six were fully immunised (four primary doses by 5 years of age), two had received three doses of vaccine, but seven proved not to have been vaccinated.[7] This underscores the importance of screening and public health intervention for infections with *C. diphtheriae* despite effective vaccination programmes.

There is a noticeable decrease in antibody levels in the population over the age of 21,[12] suggesting the need for booster doses of vaccine every 10 years during adulthood. The 16–45 year age group showed the highest incidence of non-toxigenic *C. diphtheriae* in the present study. However, it is not clear what effect, if any, revaccination might have on isolation rates of non-toxigenic strains.

Under-diagnosis of *C. diphtheriae* as the cause of acute or recurrent sore throat may occur if scientists are unaware of its importance, or if clinicians do not consider this organism a potential cause. The role of the laboratory is vital in the isolation, identification and control of diphtheria. In advanced cases of toxigenic diphtheria, laboratory confirmation often follows the clinical diagnosis; however, the disease can be difficult to diagnose.

This is especially true of cutaneous diphtheria, which is characterised by non-specific shallow ulcers anywhere on the body. They are often associated with infected insect bites and can be co-infected with other conventional pathogens, as was learned from the present study and from the published literature.[7] The potential for secondary transmission has been well documented, more often with cutaneous diphtheria than with respiratory infection.[13]

In conclusion, we would strongly recommend continued screening for *C. diphtheriae*, especially when the laboratory is situated in urban areas of high population density, such as London, which is also an important destination for travellers from all around the world. Consideration should also be given to the isolation of *C. diphtheriae* from infected tropical ulcers in patients recently returned from areas in which the disease is endemic. □

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