Infections with *Corynebacterium diphtheriae* - changing epidemiology and clinical manifestations

Report of the third international meeting of the European Laboratory Working Group on Diphtheria (ELWGD), Institut Pasteur, Paris 7 - 8 June 1996

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Abstract

A widespread epidemic of diphtheria began in 1990 in the former Soviet Union, in the context of falling immunisation rates and social disruption. Control was impeded by limited diagnostic resources in affected countries (mainly Russia) and there was a risk of spread to neighbouring countries. The European Laboratory Working Group on Diphtheria (ELWGD) was formed to assist in control of the epidemic. The ELWGD is convened by the Public Health Laboratory Service in the United Kingdom and includes 15 laboratories in Europe, one in North America (Centers for Disease Control and Prevention) and the Institute of Clinical Pathology and Medical Research, Sydney. At the group's last annual meeting in Paris, reports were presented on the progress of the epidemic, control strategies and improvements in laboratory diagnosis. The group discussed the increased carriage of, and infection with, nontoxigenic *Corynebacterium diphtheriae* in countries with high immunisation rates, including the United Kingdom and Australia. They also considered the possible relationship between this increase and the continued diphtheria outbreak in eastern Europe. Preliminary results of molecular typing of toxigenic and nontoxigenic isolates from many parts of the world were presented. It was agreed that further epidemiological investigation is required, using a standardised ribotyping system. *Comm Dis Intell* 1997;21:161-164

Introduction

In most developed countries, classical respiratory diphtheria has become rare over the past 40 years due to effective immunisation. However, the disease remains endemic in many countries, including Turkey, Bangladesh, Vietnam, Africa and some parts of South America. Cutaneous diphtheria occurs in many tropical areas, usually without causing systemic complications. Small outbreaks of potentially fatal respiratory diphtheria occur occasionally in Europe and North America, usually introduced by an imported case of either respiratory or cutaneous diphtheria.

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Table. Cases of diphtheria reported in the WHO European region, 1990 to 1995

Year	Total cases (rate per 100,000 population)	Russia (rate per 100,000 population)	Other NIS[1] (rate range)
1990	1483 (0.002)	1211 (0.82)	225 (0-0.22)
1991	3216 (0.004)	1876 (1.27)	1119 (0-2.13)
1992	5814 (0.007)	3897 (2.63)	1850 (0-3.0)
1993	19608 (0.023)	15209 (10.3)	4295 (0.03-11.8)
1994	47707 (0.055)	39582 (26.9)	8046 (0.45-32.2)
1995	50445 (0.058)	35652 (24.3)	14438 (0.81-73.0)

1. NIS - Newly independent states of the former Soviet Union

Recent significant outbreaks have occurred among skid row alcoholics in Seattle (1972-82) and in Scandinavia (1984-86) despite very high infant immunisation rates[1,2].There had previously been no indigenous cases for more than 20 years in these locations .

The largest and most widespread epidemic of diphtheria in recent times began in 1990 in countries of the former Soviet Union. More than one hundred and thirty thousand cases have been reported (Table) and the epidemic is continuing[3].

In 1993, the ELWGD was formed to:

- develop guidelines and outline future study needs and directions for laboratories;
- strengthen laboratory collaboration and support, particularly to those in greatest need;
- increase current knowledge and develop new technology relating to the laboratory diagnosis and epidemiological surveillance of *Corynebacterium diphtheriae*;
- and, importantly, to form an international network of Diphtheria Reference Centres[4].

The group includes 18 laboratories, 15 in Europe and three outside Europe including the Department of Clinical Microbiology, Institute of Clinical Pathology and Medical Research, New South Wales.

At the third international meeting of the group, at the Institut Pasteur, Paris in June 1996, the current epidemiology of diphtheria in Eastern Europe was reviewed. The group also discussed methods of laboratory diagnosis, antibiotic susceptibility, typing methods and molecular epidemiology of toxigenic and nontoxigenic *C. diphtheriae.*

Incidence of diphtheria in Europe

Dr Colette Roure, from the World Health Organization's Regional Office for Europe, reported that between 1980 and 1989 the average number of cases of diphtheria reported in Europe was 1100 (average annual rate, 0.0013 per 100,000 population). Of these more than 90% were from the USSR (average annual rate, 0.38 per 100,000 population per annum). In 1990, there was a dramatic increase. This was attributed to decreasing childhood immunisation rates, waning immunity in adults and major population movements since the dissolution of the former Soviet Union. Although all age groups were affected, the highest incidence was in adolescents and young adults. The case fatality rate was generally 5-10% but rates up to 22% were reported when there was a delay in diagnosis and treatment.

Cases of diphtheria imported from eastern Europe have been reported in a number of western European countries.

Control strategies

Control strategies recommended by WHO include:

- mass immunisation in countries where the rate is 3.5 per 100,000 population or more;
- improvement in routine immunisation rates to achieve an uptake of 95% in children and 90% in adults;
- confirmation of the diagnosis in suspected cases;
- appropriate treatment of infected individuals; and
- rapid investigation of contacts.

Implementation of these strategies, to varying degrees, has been reflected by a decrease in the number of new cases or a slowing in the rates of increase. For example following mass immunisation in Russia there was a 10% fall in cases between 1994 and 1995 which was sustained in the first few months of 1996. This compares with 2-3 fold increases in the number of cases each year for the previous three years.

Control programs have been frustrated in some countries by vaccine shortages and inadequate laboratory diagnostic facilities.

Laboratory diagnosis

Laboratory diagnosis of diphtheria involves isolation of the causative organism on selective medium (blood tellurite agar), biochemical testing to confirm the species and biovar, and tests for toxigenicity.

In some areas of the region basic laboratory skills required for the diagnosis of diphtheria had been lost. This loss of expertise was largely due to the occurrence of few cases and hence lack of experience. Basic culture media and biochemical reagents were not available in many countries affected by the epidemic. To address these problems, training workshops were held at the WHO Collaborating Center, Central Public Health Laboratory (CPHL), Colindale, United Kingdom. In addition laboratory kits were developed for distribution by WHO to laboratories. These kits contain all the basic requirements for confirmation of the diagnosis in 100 suspected cases and investigation of 1,000 contacts.

The traditional methods of detection of toxin production by *C. diphtheriae* are either the Elek test or guinea pig inoculation. Both these methods are relatively slow. Animal inoculation is expensive and, increasingly, ethically unacceptable. The traditional Elek test involves the use of a filter paper strip impregnated with diphtheria antitoxin. This is incorporated into clear agar on which the test organism(s) and

appropriate controls are inoculated at right angles to the strip. Following 48 hours incubation toxin produced by the test organism is shown by a line of precipitation which forms a line of identity with that produced by a positive control strain. In practice both false negative results (due to reduced sensitivity) and false positive results (due to nonspecific lines of precipitation) are not uncommon. A modification of this method was described by Dr Kate Engler of the CPHL, United Kingdom. This involves the use of a very thin layer of agar in small agar plates and an antitoxin-impregnated disc, around which heavy spot-inocula of test and control organisms are placed. Lines of precipitation are visible after only 16-24 hours incubation, before any nonspecific precipitation occurs. This method requires further evaluation but is potentially more rapid, accurate and economical than the conventional Elek test. It will be more accessible to laboratories in high prevalence areas, where resources are extremely limited, than newer molecular methods which are being used increasingly in Western countries.

Polymerase chain reaction (PCR) has been developed to detect phage-encoded toxin genes of *C. diphtheriae*[5-7]. Potential targets for amplification include *tox*A and *tox*B which encode toxin fragments A and B respectively and *dtx*R, which encodes an iron-dependent toxin regulatory protein. For isolates which have been identified as *C. diphtheriae,* there is generally an excellent correlation between the presence of *tox*A (the most commonly used target) and biological toxigenicity[6]. *C. ulcerans* and *C. pseudotuberculosis* are also potentially toxigenic and occasionally clinically significant. However, some other *Corynebacterium* species, without the ability to produce toxin, possess the *tox*A gene, and can give false positive PCR results. Moreover, rare biologically nontoxigenic *C. diphtheriae* strains possess the toxin gene(s) which are either repressed or defective. Although the potential clinical significance of these strains is unknown, PCR results should be interpreted with caution and only in association with the results of conventional methods of identification and toxigenicity testing.

PCR also has the potential for the detection of toxigenic *C. diphtheriae* directly in clinical specimens such as throat swabs. Dr Tanja Popovic of the Centers for Disease Control and Prevention, Atlanta described a direct PCR which is quite sensitive and can detect 150 organisms. However this method is dependent on optimisation of a number of factors, including the type of swab used, transport and storage conditions and DNA extraction method.

Antibiotic susceptibility of C. diphtheriae

The antibiotic susceptibility of 38 nontoxigenic strains of *C. diphtheriae* isolated in France between 1987 and 1993 was reported by Dr O Patey. All were susceptible to penicillin, most other commonly used β-lactams, vancomycin and perfloxacin. Two strains were resistant to lincomycin; of these one was resistant to erythromycin and the susceptibility of the other was reduced. Seven isolates (18%) were resistant to rifampicin and of these one was also resistant to erythromycin and lincomycin. Penicillin or erythromycin are the agents of choice for treatment of diphtheria or nontoxigenic *C. diphtheriae* infections. Carriers are usually treated with erythromycin. However, Dr Patey reported that eradication of carriage was more likely after treatment with rifampicin (91% after five days; 97% after seven days treatment) than with erythromycin (64% after five days, 89% after seven or ten days treatment). The value of rifampicin for treatment of carriers will depend on the degree of resistance.

Nontoxigenic C. diphtheriae infections

There have been an increasing number of reports of disease due to nontoxigenic *C. diphtheriae* in recent years, mainly in children and young adults. Outbreaks of pharyngitis have occurred among homosexual men and in educational and military establishments. Invasive infections, mainly endocarditis and septic arthritis, have been reported[1,8,9]. Most cases have been due to *C. diphtheriae* biovar *gravis.* Invasive disease is associated with significant morbidity and some mortality. Most affected individuals had been previously immunised against diphtheria, although their antitoxin levels at the time of infection were not recorded. There are no recent data available on carriage rates, but nontoxigenic *C. diphtheriae* were isolated from throat or nose swabs of 12 of 359 contacts (3%) of patients with invasive nontoxigenic *C. diphtheriae* infections in Victoria in 1994. Seven isolates were *C. diphtheriae* var *belfanti* and 5 (1.4%) were var *gravis*[9].

In the United Kingdom, 50-60% of laboratories routinely culture throat swabs for *C. diphtheriae.* Dr Androulla Efstratiou reported that the number of isolates referred to the CPHL, London, for toxigenicity testing increased from 17 in 1990 to 140 in 1995. Seventy-five per cent of these were *C. diphtheriae* var *gravis*. To determine the clinical significance of these isolates, a questionnaire was sent to referring doctors and laboratories. Most isolates were from throat swabs of children and young adults with severe pharyngitis which had not responded to, or recurred after treatment with, penicillin. Most responded to therapy with erythromycin. The greatest proportion of isolates were from general practice or genitourinary medicine clinics and, in most cases, no other potential pathogen had been isolated although viral cultures had rarely been done. There have been few cases of invasive infection in the United Kingdom.

The significance of these findings is not clear. Mechanisms of pathogenicity of nontoxigenic *C. diphtheriae* are poorly understood. The organism clearly is potentially invasive in a minority of individuals, many of whom have underlying risk factors, such as intravenous drug use or cardiac valvular disease[8]. It is known that nontoxigenic *C. diphtheriae* can regain toxigenicity by lysogeny with the phage carrying the toxin gene and it is postulated that this can occur in vivo[10]. Dr G Tseneva of the Pasteur Institute, St Petersburg reported that some *C. diphtheriae* isolates from long-term carriers, which were nontoxigenic by Elek test and rabbit inoculation, were shown by PCR to contain *tox*A. After repeated passaging on Elek medium, which contains a low iron content to inhibit the toxin repressor protein (DtxR), 50% of these isolates produced toxin. Thus, nontoxigenicity is apparently sometimes due to reversible toxin gene repression, rather than loss of the gene or the carrier phage.

There have been a relatively large number of cases of invasive nontoxigenic *C. diphtheriae* infections in Australia recently. This includes at least seven in New South Wales, one each in Queensland and Western Australia and three in Victoria.[8, 9, 11] It is therefore likely that throat carriage or infection is not uncommon but remains undetected because most laboratories do not culture throat swabs from patients with sore throats for *C. diphtheriae*.

Molecular typing of C. diphtheriae

A variety of methods for the epidemiological typing of *C. diphtheriae* isolates have been described. These include ribotyping and pulsed field gel electrophoresis (PFGE)[11,12]. They have been used to demonstrate predominant ribotypes among toxigenic isolates of both *C. diphtheriae* var *gravis* and var *mitis* from Russia and surrounding countries. They have also been used to trace the origin of imported cases in western Europe[12].

Multiple clones of nontoxigenic *C. diphtheriae* var *gravis,* with one predominating (six of seven isolates from cases in New South Wales), were shown to have caused invasive infections in Australia[11]. PFGE was used to demonstrate similarity between the New South Wales isolates and those from three patients with endocarditis and five of their contacts in Victoria[12]. Dr Aruni DeZoysa (CPHL, London) reported that, among 118 nontoxigenic *C. diphtheriae* var *gravis* isolates referred to the CPHL in 1995, there were 23 different ribotypes. However, 75% belonged to a single ribotype which, on the basis of preliminary results, appears to be very similar to a ribotype found among isolates from Eastern Europe.

Unfortunately, because different endonucleases, probes and ribotype nomenclature are used, the results of one study cannot be compared with those of another. It was therefore proposed by Professor Patrick Grimont of the Institut Pasteur, Paris that a standard ribotyping method and common nomenclature be adopted. This would enable the establishment of a database of ribotypes, validated using appropriate computer software. It would also assist in the international surveillance of outbreaks of diphtheria and nontoxigenic *C. diphtheriae* infections, contribute to a better understanding of the epidemiology of this disease and improve disease control worldwide.

Ribotyping and PFGE of *C. diphtheriae* are being performed at the ICPMR, Westmead. Ribotyping will be standardised with the international method once this has been established. However, in a recent comparison of the two methods using 100 toxigenic and nontoxigenic isolates of *C. diphtheriae*, we found that PFGE was significantly more discriminatory than ribotyping (K Cheung and L Gilbert, unpublished data).

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