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CORYNEBACTERIUM DIPHTHERIAE INFECTIONS CURRENTLY AND IN THE PAST

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ABSTRACT

Along with the introduction of common obligatory vaccinations against diphtheria, the disease has been limited in developed countries. However, diphtheria is still endemic in developing countries. Due to a growing popularity of visiting these countries, there is a risk of importation of the disease to Europe. Studies revealed that over 60% of persons aged > 40 years in the Polish population do not have a protective level of antibodies against diphtheria. Furthermore, an access to diphtheria antitoxin, which is essential in diphtheria treatment, is now hardly accessible in Europe.

On the other hand, in many countries, including Poland, new infections caused by non-toxigenic *Corynebacterium diphtheriae* have been emerged. Such infections are frequently manifested by bacteraemia and endocarditis with a high fatality rate, amounting even to 41%.

Key words: *diphtheria, Corynebacterium diphtheriae, invasive infection, vaccination*

PATHOGEN CHARACTERISTICS

Corynebacterium diphtheriae is a Gram-positive, aerobic, pleomorphic coccobacillus, frequently with club-shaped edges. Based on the colony morphology and biochemical properties, four *C. diphtheriae* biotypes were described: *gravis, mitis, intermedius* and *belfanti* (1, 2). Until recently, strains capable of producing diphtheria toxin were exclusively considered to be pathogenic for humans. *C. diphtheriae* acquires the potential to produce diphtheria toxin through the lysogenization with corynebacteriophage carrying *tox* gene. Recently, severe infections caused by non-toxigenic strains are also reported. Its course is considerably different compared to diphtheria (3).

Irrespective of the fact that pathogens belonging to *Corynebacterium* are prevalent in environment – soil, plants, skin and mucosa of humans and animals - *C. diphtheriae* is present nearly only in humans. Recently, however, it was observed that horses and other domestic animals, including cats and dogs, may also be the carriers of this pathogen (4-6).

INFECTIONS CAUSED BY TOXIGENIC CORYNEBACTERIUM DIPHTHERIAE STRAINS

Toxigenic *C. diphtheriae* strains cause the disease called diphtheria. Depending on the anatomic site involved, there are the following manifestations of diphtheria: pharyngeal, laryngeal, aural, nasal, cutaneous, conjunctival, umbilical and genital. The disease is transmitted through respiratory droplets or direct contact with an infected person or carrier, their secretions or objects that were in contact with the infected person or carrier. During the course of diphtheria bacteria colonize locally the mucosa. Usually, they do not permeate the tissues, however, the toxin, which is produced by these bacteria, is absorbed into the bloodstream and distributed throughout the whole organism. Pharyngeal and laryngeal diphtheria are the most common manifestations of this disease. Following a short period of incubation, lasting for 2-5 days, fever and sore throat are present. At the site of colonization on the mucosa of the pharynx and larynx, necrotic membranes appear, i.e. pseudomembranes which are grey, translucent or black-coloured. Any efforts to remove it cause bleeding. Simultaneously, lymph nodes are enlarged. Neck's size is increased (called

bull neck, proconsul neck or Neron neck). Formation of pseudomembranes and considerable enlargement of lymph nodes result in the narrowing of the pharynx and larynx lumen. Consequently, it hinders swallowing and breathing. Toxin, which is produced by *C. diphtheriae,* permeates the bloodstream and all organs. It causes an early damage to the fibres of cardiac muscle and its inflammation, conduction disorders and, possibly, heart block as well as demyelination of nerves which leads to the paralysis of the palate and ocular muscles. Paralyses similar to those observed in case of the Guillain-Barré syndrome may also be present (7, 8).

Nasal, aural, conjunctival, cutaneous, umbilical and genital diphtheria occures due to the colonization of toxigenic *C. diphtheriae* strains on localized areas such as wounds, abscesses, skin lesions. At these sites, inflammation is developed, accompanied by serosanguineous exudate, toxin production and formation of necrosis and pseudomembranes (7).

Diphtheria toxin is a potent toxin whose lethal dose for susceptible species (i.a. human, monkeys, rabbits, guinea pigs) was determined at 100-150 ng/kg of body mass (9). Thus, a basic therapy of diphtheria consists in the neutralization of diphtheria toxin circulating in organism by administering appropriate doses of antitoxin. It neutralizes only unbound toxin, i.e. toxin which has not fixed to the host organism cells, thus, early initiation of therapy with antitoxin is of importance (10). Unfortunately, diphtheria antitoxin is now hardly accessible in both Europe and America as the majority of producers stopped to manufacture it. Only few producers worldwide with examples being Microgen (Moscow, Russia) and Vins Bioproducts (Hyderabad, India) still produce diphtheria antitoxin (11, 12).

DIPHTHERIA IN POLAND

In 1919-1937, the number of diphtheria cases and deaths due to this disease ranged from 1815 to 23 470 and 219 to 1 186, respectively. At the time of occupation, no unified register of infectious diseases was held on the territory of Poland. Immediately after the Second World War, i.e. 1945-1949, the number of cases varied from 13 713 to 22 510, including from 600 to 1 494 deaths. In 1950-1956, a large diphtheria epidemic was present in Poland. During its peak, the number of cases ranged from more than 24 000 to nearly 44 000, while the number of fatal cases varied from 1 600 to more than 3 000 annually (13, 14). The number of cases decreased considerably after introduction of common vaccinations against diphtheria in the whole country in 1954, (Figure 1). In 1981-2000, only single infections were reported. Since 2001 up to the present time, no diphtheria cases were reported in Poland (14, 15).

It is worth to note that Poland was one of the first countries in Europe to introduce vaccinations against diphtheria. In 1930, diphtheria vaccinations were held in Warsaw, Łódź and Vilnius, and then, they were introduced to other regions of the country. During the Second World War, no diphtheria vaccinations were held. Following the WWII , vaccinations were not conducted in a systematic manner. Furthermore, only one dose of vaccine was mainly administered. It was not until the end of 1954, when the Ministry of Health commenced the mass vaccinations. All children aged 4 months to 7 years were subject to obligatory vaccinations. Primary immunization schedule included the administration of three doses of vaccine at specific intervals, and then, booster doses every 3-4 years (13).

DIPHTHERIA WORLDWIDE

A number of countries in Africa, South America, Asia, South Pacific, Middle East, Eastern Europe as well as Haiti and the Dominican Republic remain to be endemic areas for diphtheria (16). In Europe, the largest diphtheria epidemic in the recent time was reported in the countries of the former USSR in the 90s of the last

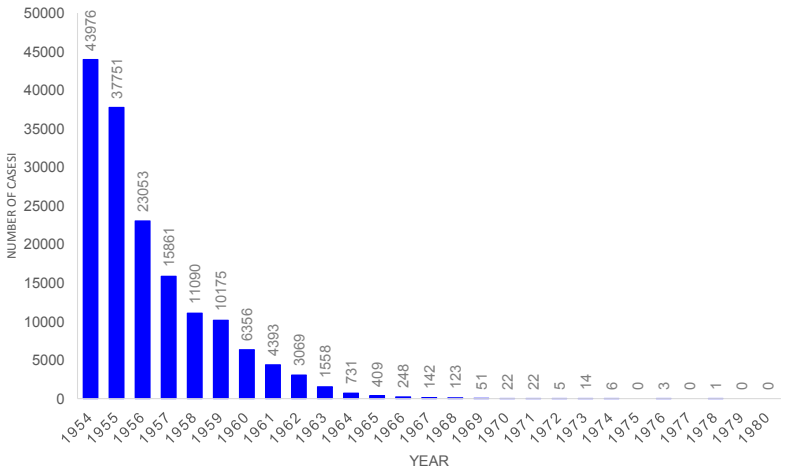

Fig. 1. Number of diphtheria cases in Poland in 1954-1980 (Gałązka A) (14).

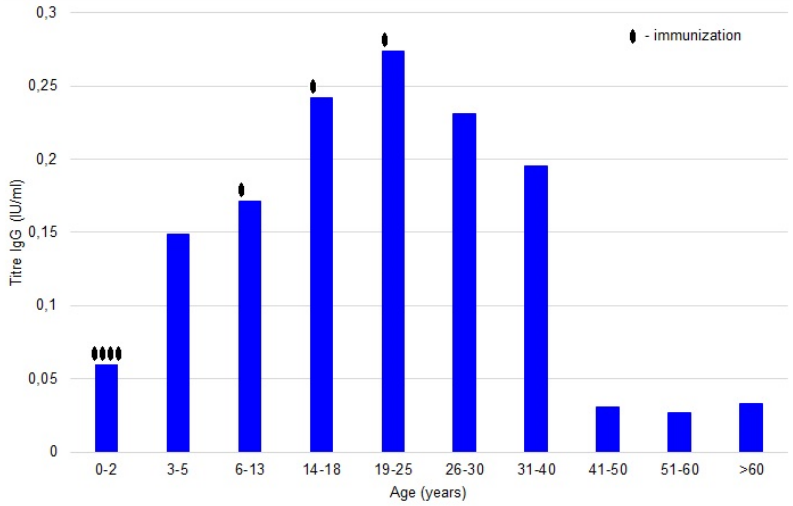

Fig. 2. Geometric mean of anti-diphtheria toxin antibody titre in Polish population by age groups (23).

century. In the peak of the epidemic in 1995, a total of 50 425 cases and nearly 1 500 deaths were registered (17). Overall, in 1990-1996, more than 150 000 infections and nearly 4 500 fatal cases were noted (14). In the present time, diphtheria occurs sporadically in the developed countries. According to the data of the World Health Organization, a total 4 680 of diphtheria infections were reported in 2013 worldwide. Based on the ECDC data, 20 diphtheria cases were notified in 2011 in Europe. These cases were reported in Latvia (6 cases), France (5 cases), Germany (4 cases), Sweden (4 cases), Great Britain (2 cases) and Lithuania (1 case). It is worth to note that the highest number of diphtheria cases in Europe in the recent years is reported in Latvia which is considered to be an endemic area for diphtheria, e.g. in 2007 and 2008 a total of 15 and 28 cases were registered there, respectively, while the total number of diphtheria infections in Europe was 21 in 2007 and 42 in 2008 (18).

Epidemiological data suggest that a more common cause of diphtheria in developed countries is not *C. diphtheriae*, but *C. ulcerans* which also has the potential to produce diphtheria toxin. For example, in 2000-2009, 43 toxigenic *Corynebacterium* strains were isolated from patients in Great Britain. Of them, 27 (63%) were *C. ulcerans* (19). In France, 12 *C. ulcerans* strains (63%) were isolated from 19 cases infected with toxigenic *Corynebacterium* in 2002-2008 (20). Having considered the ECDC data on diphtheria in Europe in 2011 presented above, 7 cases were caused by *C. ulcerans*.

IMMUNITY TO DIPHTHERIA IN POLISH POPULATION

In the past, diphtheria was considered to be a childhood disease as it was of the highest incidence and fatality in this group (14). Widespread vaccinations against diphtheria resulted not only in a decrease of incidence, but also a shift of incidence to the elder age groups as a lack of contact with this pathogen prevented from acquiring the active immunity through being repeatedly exposed to the infection with toxigenic *C. diphtheriae* (21).

Current obligatory immunization schedule in Poland indicates to administer 7 doses of vaccine against diphtheria at the age of 2, 3-4, 5-6, 16-18 months and then 6, 14 and 19 years (22). In case of adults, it is recommended to be given a booster dose every 10 years. According to the WHO data, 96-99% of children are given primary vaccinations against diphtheria in Poland. Study conducted by *Zasada* et al. (23), however, suggest that only 64% of children aged up to 5 years have a protective level of antibodies against diphtheria. Along with the administration of successive doses of vaccine, the percentage of immunized persons increases. Its value is the highest in the 19-25 age group, i.e. following the administration of the last obligatory does of vaccine against diphtheria. In this group, nearly 83% of tested individuals had a protective level of anti-diphtheria toxin antibody. A dramatic decrease in the anti-diphtheria toxin antibody titre was demonstrated in persons aged > 40 years, where only 36% had antibody titre which ensures a basic protection. None of them had antibody titre giving complete and long-term immunity against diphtheria (23). Figure 2 presents a geometric mean of anti-diphtheria toxin antibody titre in different age groups of the Polish population.

INFECTIONS CAUSED BY NON-TOXIGENIC CORYNEBACTERIUM DIPHTHERIAE STRAINS

Until recently, non-toxigenic *C. diphtheriae* strains were not considered to be pathogenic. In Europe and

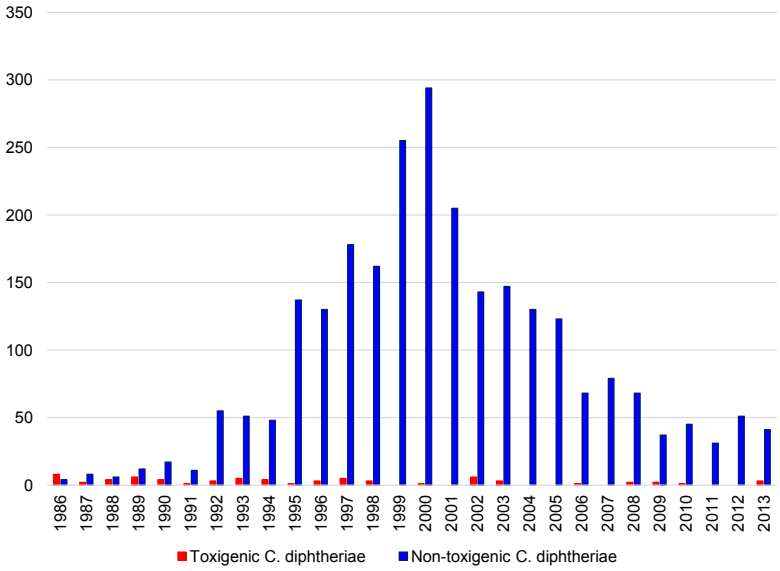

Fig. 3. Number of toxigenic and non-toxigenic *C. diphtheriae* strains isolated in England and Wales in 1986-2013 (25).

America, however, serious invasive infections caused by these pathogens began to be reported in the 90s of the last century. Such cases were reported, i.a. in France, Italy, Switzerland, Germany, Great Britain, Brazil, Canada as well as Poland (4, 24). The most spectacular increase in the number of infections caused by non-toxigenic *C. diphtheriae* was reported in England and Wales where 8 toxigenic strains and 1 non-toxigenic strain were isolated in 1986. Then, the number of isolated non-toxigenic strains began to increase dramatically in the 90s while in 2000 it amounted to 294. At that year, only one toxigenic strain was isolated (25, 26). Nowadays, the number of non-toxigenic *C. diphtheriae* strains isolated in England and Wales annually amounts to several dozens (Fig. 3).

In Poland, the first case of bacteraemia and endocarditis due to non-toxigenic *C. diphtheriae* strain was reported in 2004. Since that time, such cases are noted every year (3, 27). Figure 4 shows non-toxigenic *C. diphtheriae* cases whose isolates were sent to the Department of Bacteriology of the NIPH-NIH for verification. There is no obligation to notify the infections caused by non-toxigenic *C. diphtheriae* strains, thus, it may be presumed that the number of such cases is higher.

The phenomenon observed suggests that non-toxigenic *C. diphtheriae* strains acquired the potential to permeate the tissues. It is worth to note that diphtheria vaccine contains diphtheria toxoid, thus, it prevents from the action of diphtheria toxin, however, it does not protect against the infection with non-toxigenic strains. Studies demonstrate that the homeless, persons addicted to alcohol, people who inject drugs, individuals suffering from diabetes, cirrhosis and those having massive dental caries are at a risk of invasive infection with non-toxigenic *C. diphtheriae* strains. It is assumed that dental caries may be a portal of entry for invasive infection with *C. diphtheriae* (3). In cases of invasive infections with non-toxigenic *C. diphtheriae* strains, fatality rate is very high and amounts to 36-41% (28, 29).

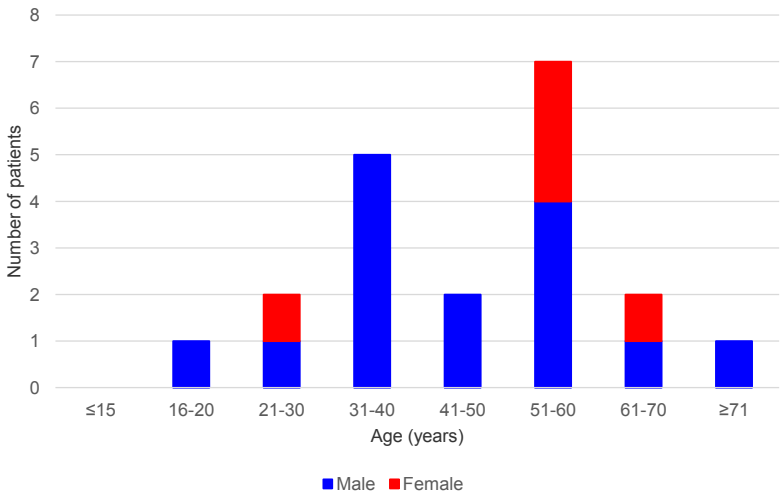

Fig. 4. Number of non-toxigenic *Corynebacterium diphtheriae* infections in Poland in 2004–2012 (excluding 5 cases for whom age is not available) (3).

SUMMARY

Low antibody titre in persons aged > 40 years constitutes a risk of introduction of diphtheria from its endemic areas, which are becoming more popular tourist destinations. Simultaneously, current political situation in the eastern part of Europe, may result in an increased risk of the occurrence of diphtheria in Poland and Western European countries. Such situation was observed in the 90s of the 20th century. It resulted from a collapse of the immunization system and increased migration of population. Thus, administration of diphtheria booster doses to adults is of importance. A special attention should be paid to persons caring for cats as they were identified to be a possible source of infection with toxigenic *C. ulcerans* and *C. diphtheriae* strains in the recent years. In order to facilitate the physicians and patients the process of diphtheria vaccination monitoring in adults, it should be considered to make the recommendations more precise by, e.g., indicating administration of booster doses at the age of 30, 40, 50 year etc. instead of general recommendations stating that booster doses should be given every 10 years.

An attention should be also paid to the occurrence of invasive infections with non-toxigenic *C. diphtheriae* strains. Currently, little is know about the pathogenesis and epidemiology of such infections. Probably, infections caused by non-toxigenic *C. diphtheriae* strains should also be subject to obligatory notification. It would allow not only for determining the actual number of these infections, but also conducting reliable epidemiological studies.

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