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DIPHTHERIA IN A VACCINATED ADULT IN RIO DE JANEIRO, BRAZIL

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SHORT COMMUNICATION

ABSTRACT

In 1999, a case of diphtheria in a 32-year-old woman was reported. The patient developed a sore throat immediately after participating of a five-day meeting with European workers in Rio de Janeiro. Her history included complete pediatric immunization (DTP) and three doses of adult formulation tetanus and diphtheria toxoid (dT) two years earlier. Clinical diagnosis of diphtheria was not made until microbiologic examination of specimens confirmed toxigenicity of *Corynebacterium diphtheriae* var. *gravis*, a biotype currently found circulating within Europe where diphtheria remains epidemic. This case reinforces the potential susceptibility of Brazilian adults to epidemic diphtheria in the vaccine era.

Key words: *Corynebacterium diphtheriae*, dT immunization, adult diphtheria patient

Epidemic diphtheria may occur despite fairly high levels of childhood coverage. The worst epidemic of diphtheria in postvaccination era (≥ 157,000 cases and 5,000 deaths) demonstrated conclusively the potential susceptibility of adults to epidemic diphtheria in the vaccine era. In 1990, diphtheria reemerged in the Russian Federation and spread to all Newly Independent States (NIS) with a high proportion of cases in adults, 64% to 82%, respectively. Severe disease and a high percentage (30%) of fatal cases were documented among vaccinated individuals (2,14).

The spread of epidemic seemed facilitated by large scale population movements; socioeconomic instability, partial deterioration of health infrastructure; delay in implementing measures to control epidemic; inadequate information for physicians and the public; lack of adequate supplies for prevention and treatment in most of the countries (2,8).The reasons for reemergence of epidemic diphtheria in countries where immunization programs had nearly eliminated diphtheria are not fully understood but are thought to include the introduction of toxigenic *C. diphtheriae* strains of a new biotype into the general population, besides low coverage with diphtheria vaccine among children and large gap of immunity among adults (4,22).

Other factors besides antitoxin protection seem to influence vulnerability of diphtheria, namely, the dose and virulence of the diphtheria bacilli involved, as well as the general immune status of the person infected.

Diphtheria is becoming subject of renewed interest owing to cases epidemiologically related to immunodeficient states, including Human Immunodeficiency Virus (HIV) infections (8,10,21).

The occurrence of cases of diphtheria among immunized persons and the variety in the adhesive properties of *C. diphtheriae* points to the importance of other factors as well (8,14,19).

We report a case of respiratory diphtheria caused by toxigenic *C. diphtheriae* var. *gravis* strain in a fully immunized adult with known recent permanent contact with European travelers.

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On June 14th, 1999, a previously healthy 32-year-old woman, lawyer, residing in Rio de Janeiro, Brazil was examined in an outpatient clinic with sore throat, difficulty swallowing, fever (37-38ºC) and profound prostration. She worked in a foreign company and had known permanent contact with European travelers during the last five days (June 7-11th) before onset of symptoms. On physical examination she had a whitish membrane covering both tonsils. Therapy was initiated with 500mg of oral erythromycin (8/8h) after collection of pharyngeal swabs for culture in a local laboratory. On the morning of June 15th the symptoms worsened and she was hospitalized. Multiple complications had developed a few hours after admission including pulmonary edema and renal failure. Cardiologic examination, including repeated ECGs and echocardiography and neurologic examination performed during hospitalization showed no signs of affection. Gram-positive, nonmotile, aerobic, catalase-positive bacilli consistent with *C. diphtheriae* were sent to the Diphtheria Reference Laboratory at Faculdade de Ciências Médicas, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, RJ, Brazil, for further confirmation of species and toxigenicity testing. Diagnosis of diphtheria was not made until throat culture yielded growth of a toxigenic strain of *C. diphtheriae* on June 16th. Diphtheria antitoxin was then administered and symptoms began to improve. The patient was discharged on June 23rd.

Specimens obtained from throat and nasopharynx from over 50 family, community and working contacts exposed to the case-patient around the time of illness were also investigated.

Clinical specimens were cultured in 5% defibrinated sheep blood agar, tellurite chocolate agar and thioglycollate broth incubated at 37ºC for 24/48h. *C. diphtheriae* grew predominantly and in association with pinpoints β-hemolytic streptococci colonies during primary culture of the respiratory tract specimens on local laboratory. The methods used for biochemical profiling of diphtheria bacilli have been outlined in detail before (6,10,11,18).

Briefly, the suspect colonies grown on tellurite chocolate agar and/or blood agar plates were inoculated on King B medium for porfirin production testing (fluorescence test). Microorganisms were also submitted to the double sugar-urease (DSU) test (test for glucose and maltose utilization and urease activity). Tests of nitrate reduction, pirazynamidase activity (PYZ) and CAMP-reaction, were used to differentiate among corynebacteria. Toxigenicity testing was made by toxin-antitoxin precipitation methods (Radial Immunodiffusion Test and Elek Test). Toxigenic *C. diphtheriae* var. *mitis* (CDC- E8392) from Centers for Disease Control and Prevention, Atlanta, Georgia, USA was used as control. The minimal inhibitory concentrations (MIC) of penicillin and erythromycin antimicrobial agents was determined by the E-Test (17).

Bacteriological and toxigenicity tests demonstrated that the strain isolated from throat of the adult patient represented toxigenic *C. diphtheriae*. Microorganisms were tellurite reductase positive and produced fluorescence under ultraviolet light; The biochemical reactions were: catalase and nitrate positive; nonmotile; urea and aesculin hydrolysis negative; fermentation of glucose, maltose and starch positive; pyrazinamidase and CAMP reaction negative. Antimicrobial susceptibility test revealed resistance to penicillin G (MIC 0.125 μg/ml) and susceptibility to erythromycin (MIC 0.016 μg/ml) as previously observed in 1971 (13).

The strain was identified as *C. diphtheriae* var. *gravis,* a biotype currently found circulating within Europe and responsible for most diphtheria cases during the epidemics. Prolonged exposure at close proximity or multiple exposures over time with European travelers could have probably favored the acquisition of the organism. Data allowed us to hypothesize that this toxigenic *C. diphtheriae* var *gravis* strain was introduced into Brazil from Europe. However, examination of close contacts from family, community, and working company, did not reveal any carriers of diphtheria bacilli. Further characterization by ribotyping would be necessary to demonstrate if this isolate produces a molecular pattern similar to the "epidemic pattern" currently circulating within Eastern Europe.

At present, during the Vaccine Era, most physicians have little experience in diagnosing and treating diphtheria. The resurgence of diphtheria after a long period of absence contributed to a lack of awareness in the medical community and the general public about the rapid and often fatal course of diphtheria. In Kyrgyzstan, a recent investigation identified delays in seeking health care, diagnosing the illness, and initiating appropriate therapy. About 40% of patients had a previous consultation with a health professional but only half of these patients were hospitalized on the day of initial presentation. The remainder were treated as outpatients (usually with a diagnosis of severe sore throat) or hospitalized some days later. Delay in seeking medical care and adequate diphtheria treatment was associated with fatal outcome (15).

Antibiotics are useful in eradicating the organism and thereby limiting both toxin production and transmissibility. However, diphtheria antitoxin should be administered promptly based on the clinical presentation and presumptive diagnosis and cannot wait for laboratory confirmation. Delay in initiating antitoxin treatment is associated with increased incidence of myocarditis, paralysis and death (4,7,16).

Despite the shift to an older median age among patients, the clinical features of diphtheria in the 1990s still similar to those observed in the Prevaccine Era. In many advanced cases of diphtheria, the clinical diagnosis would normally precede microbiologic diagnosis. However, its sometimes often difficult to diagnose *C. diphtheriae* infections clinically, especially in cases without local pseudomembrane formation or with coinfections including pneumonia or bronchitis (4,16,23) or in cases of endocarditis and other invasive forms of disease (18).

The present case illustrates the often difficulty to diagnose diphtheria clinically. Clinical diagnosis of diphtheria and antitoxin administration was not made until microbiologic examination of specimens confirmed growth of toxigenic *C. diphtheriae* within 36 h of receipt of the isolate by the Reference Laboratory. The primary care physician in the present case was indeed fortunate since the patient had diphtheria; the results could have been tragic.

The rarity of cases and the expense and complexity associated with laboratory diagnosis provided many countries with the indication to cease screening clinical specimens for *C. diphtheriae*. Delay between isolation of a suspicious organism and the results of toxigenicity tests can provoke great anxiety among laboratory staff, clinicians, and public health officials. In the United Kingdom and the United States, it is recommended that all suspect isolates be referred, without delay, to the Reference Laboratory and Centers for Disease Control and Prevention (CDC) Diphtheria Laboratory, respectively. These systems seem to be working extremely well. This highlights the importance of reference facilities within countries (1,3).

Unfavorable economic conditions presented by most regions of the Brazilian vast territory turn difficult the remittance of suspect isolates for bacteriological confirmation and toxigenicity tests to Reference Laboratories. At present, Elek facilities are still not available in many laboratories throughout the country.

Since diphtheria remain endemic in Brazil, expertise and recognition of the organism should not decline. The goal of the laboratory in the diagnosis of diphtheria is to provide simple, rapid, and reliable methods to assist clinicians in achieving the correct diagnosis. The laboratory may also aid the clinician by eliminating suspected cases or contacts from further clinical investigation, treatment or control measures. The screening assays based on the fluorescence and the double sugar-urease tests developed for differential diagnosis of *C. diphtheriae* are practical procedures and economic for laboratory diagnosis (5,6,11) also during investigation of carriers of nontoxigenic bacilli in skin lesions or respiratory tract (12,20).

Previous studies allowed us to suggest the Brazilian laboratories to consider all the fluorescent and maltose-positive Gram-positive rods isolated from diphtheria suspected cases or close contacts as *C. diphtheriae*. The fluorescence test eliminated the need for additional identification tests in 70% coryneform bacteria (nonfluorescent strains) while most of the fluorescent and maltose-positive Gram-positive bacilli were identified as *C. diphtheriae* (5,20).

Despite the success of mass immunization in many countries diphtheria remains a serious health problem within many regions of the world (2,10,11,14,15,22,23). Diphtheria should be suspected in any patient who lives in an endemic area and that clinically significant outbreak of diphtheria could occur in the future. Increasing international travel and the emergence of epidemic clones present a threat to Brazil and require achieving and maintaining high coverage with diphtheria toxoid-containing vaccines in both children and adults. Our investigation substantiates the importance of maintaining appropriate experience with this important pathogen in order to avoid future problems and guarantee continued vigilance by laboratories and medical community.

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RESUMO

Difteria em adulto vacinado no Rio de Janeiro, Brasil

Um caso de difteria ocorreu, em 1999, em mulher de 32 anos de idade. A paciente iniciou episódio de dor de garganta imediatamente após participação em reunião com profissionais europeus durante cinco dias consecutivos, no Rio de Janeiro. Ela declarou ter sido submetida ao esquema completo de imunização contra difteria (DTP-tríplice bacteriana) na infância e a doses de reforço (dT-dupla adulto) há dois anos. O diagnóstico clínico da doença só foi firmado após o laboratório de microbiologia ter confirmado a capacidade de produção de toxina pela amostra isolada de *Corynebacterium diphtheriae* var. *gravis*, biotipo não fermentador de sacarose comumente encontrado em diversos países europeus e responsável pela atual epidemia na região correspondente a antiga União Soviética. Na era da vacinação antidiftérica, indivíduos adultos de nossa comunidade podem apresentar-se potencialmente susceptíveis a difteria.

Palavras chave: *Corynebacterium diphtheriae*, imunização dT, difteria em adulto

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