Corynebacterium propinquum bronchopneumonia in a child with ataxia telangiectasia

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Nondiphtherial *Corynebacterium* species isolated from clinical specimens are usually considered as contaminants by many clinicians when reported by microbiologists. However, an increasing number of studies have confirmed the importance of *Corynebacterium* spp. in the etiology of a variety of infectious processes. In this report, we present a case of bronchopneumonia caused by *Corynebacterium propinquum*. The infection occurred in a seven-year-old child who had a history of immunosuppression due to ataxia telangiectasia. The purulent sputum of the patient yielded a large number of polymorphonuclear leucocytes with abundant gram-positive coryneform bacilli in gram staining and pure growth of coryneform bacteria in culture. Definitive identification as *C. propinquum* was made by matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) and 16S rRNA gene sequencing. *C. propinquum* should be recognized as a potential pathogen and included in the etiologic diagnostic algorithm, particularly in patients with immunosuppressive conditions.

Key words: Corynebacterium propinquum, bronchopneumonia, gram staining, child, ataxia telangiectasia.

Corynebacterium species other than *Corynebacterium diphtheriae* are ubiquitous in nature and commonly colonise the skin and mucous membranes of humans and other mammals. Their pathogenic potential has been questioned. During the last decade there have been a number of reports associating coryneform bacteria with human infections, such as bacteremia, endocarditis, osteomyelitis, lower respiratory tract, eye and genitourinary infections. With the duration and intensity of immunosuppression that patients are now subjected to and the increased use of indwelling intravenous devices, the role of coryneform bacteria has become more significant than in the past[1,2].

Corynebacterium propinquum (*C. propinquum*) is a member of normal bacterial flora colonizing the oral cavity. In some rare cases, *C. propinquum* is reported as an emerging pathogen of the respiratory tract especially in patients with chronic pulmonary disease and immunosuppression[1]. Here we describe a case of a *C. propinquum* bronchopneumonia in an immunocompromised child in Turkey.

Case Report

A seven-year-old girl was admitted to the pediatric allergy and immunology clinic of Şişli Hamidiye Etfal Training and Research Hospital in September 2015, with complaints of productive cough and purulent sputum. She had a history of immunosuppression due to ataxia telangiectasia and also had been diagnosed as asthma. The patient was receiving inhaled corticosteroid and intravenous immunoglobulin replacement treatment periodically. Five weeks before admission, she had been hospitalized

for pneumonia however no respiratory samples were sent for culture. She had recovered after treatment with intravenous ceftriaxone. At the time of admission, the patient had no fever and her ventilation was spontaneous, with mild tachypnea. Chest auscultation revealed widespread inspiratory and expiratory crackles and rhonchi. A chest X-ray showed bilateral peribronchial infiltration (Fig. 1A). Blood investigation showed hemoglobin level of 12.7 g/dl, leucocyte count of 9960/μl with 6360/μl neutrophils, platelet count of 549000/μl. C-reactive protein and procalcitonin levels were 11.5 mg/L and 0.04 ng/ml, respectively. The patient was hospitalized in the pediatric infectious disease department with the diagnosis of bronchopneumonia. After sputum and blood samples were taken for microbiological analysis, empirical treatment with intravenous piperacillin-tazobactam 300 mg/kg/day (divided into four doses) was started. On the sixth day of treatment the patient did not improve. For differential diagnosis, contrast-enhanced computed tomography of thorax was performed and showed centrilobular nodules with tree in bud appearance at lower lobe of the left lung (Fig. 1B). Infective process was compatible with defined lesions and investigation for atypical agents was offered.

Direct microscopic examination of the sputum revealed a great number of polymorphonuclear leucocytes with numerous intracellular and extracellular gram positive bacilli showing typical coryneform alignment. In addition, the red strands of mucus in the background was observed indicative of a qualified sputum specimen (Fig. 1C). Sputum culture showed a heavy, pure growth of tiny colonies on sheep blood agar and chocolate agar and no growth on MacConkey agar after 24 hours of incubation at 37°C. A further 24 hours incubation produced creamy, round, whitish, non-hemolytic, catalase positive colonies measuring approximately 1 mm in diameter. Gram stain from culture revealed gram positive rods which have characteristic V-shaped or Chinese letters arrangements. The isolate was analyzed by MALDI-TOF MS on a Microflex LT (Bruker Daltonics, Bremen, Germany) platform and identified as *Corynebacterium propinquum* with an identification score of 2.20. In a second step, the isolate was further tested by BD Phoenix bacterial identification system (BD Diagnostic Systems, Sparks, MD, USA) and identified as *Corynebacterium pseudodiphtheriticum* with a set of biochemical characteristics including presence of urea hydrolysis and absence of carbohydrate fermentation and esculin hydrolysis.

To confirm bacterial identification at species level, 16S rRNA sequence analysis was performed under previously described conditions[3]. The 16S rRNA gene sequence was compared with all eubacterial 16S rRNA sequences available in the GenBank database by using the BlastN software (http://www.ncbi.nlm.nih.gov/BLAST/). The sequence had a 99% similarity to that of *C. propinquum*.

Antimicrobial susceptibility testing of the isolate was performed for rifampicin, gentamicin, ciprofloxacin, tetracycline, and clindamycin on Mueller-Hinton Fastidious agar by using disk diffusion method according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria[4]. Penicillin, cefotaxime, ceftriaxone, imipenem, daptomycin, and vancomycin susceptibilities were determined according to the Clinical and Laboratory Standards Institute (CLSI) criteria by using the Etest method[5]. The isolate was susceptible to all antibiotic agents tested except clindamycin.

Since the patient did not show clinical improvement during this period, vancomycin 40 mg/kg/day (divided equally into four doses) was added to the treatment. Investigations for tuberculosis were all found to be negative. There was no growth in blood culture. Clinical condition of the patient improved with vancomycin treatment. At a 2-week follow-up visit, she was completely asymptomatic, and was discharged on 14th day of piperacillin-tazobactam and 10th day of vancomycin treatment.

Discussion

Estimating the clinical significance of nondiphtherial *Corynebacterium* species isolated from clinical specimens is often confusing for clinicians and clinical microbiologists. This is in part due to the natural habitat of coryneform bacteria, which may lead to their recovery as a part of the normal flora if specimens were not taken correctly. The predominant appearance of coryneform bacilli and polymorphonuclear leukocytes on direct microscopical examination

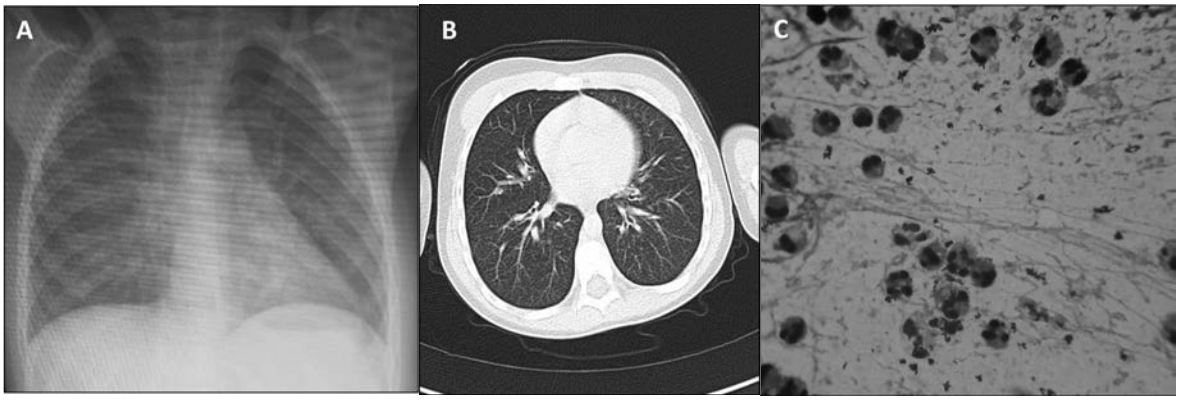

Fig. 1. (a) A chest radiograph of the patient showing bilateral peribronchial infiltration. B: Contrast-enhanced computed tomography of thorax. C: Gram-stain of the sputum specimen showing a great number of polymorphonuclear leucocytes with numerous intracellular and extracellular coryneform bacilli.

of Gram-stained respiratory specimens, together with the growth of *Corynebacterium* in pure or abundant culture, suggests a pathogenic role for these bacteria[1].

C. propinquum (formerly called CDC coryneform group ANF-3) is considered a member of the normal oropharynx and skin flora. It has occasionally been involved in opportunistic infections such as respiratory infections[6-8], bacteremia[9], endocarditis[10], pleural effusion[11], osteoarticular infections[12,13], trichomycosis axillaris[14], non-gonococcal urethritis[15], and contact lens related keratitis[16]. Previous hospitalization, immunosuppression, chronic corticosteroid therapy, previous broad-spectrum antibiotic therapy, and underlying respiratory disease are the risk factors shared in most of the published cases of *C. propinquum* infections. Similar to these, our patient had a history of immunosuppression due to ataxia telangiectasia and had been receiving inhaled corticosteroid for asthma. In addition, she had been hospitalized and received antibiotic treatment five weeks before admission to the hospital.

It is known that patients with chronic respiratory infections have a persistent and non-innocent colonization of the lower respiratory tract by several non-pathogenic microorganisms, among which non-diphtherial *Corynebacteri* are included. The selective pressure caused by previous antimicrobial treatment favors the opportunistic overgrowth of these bacteria in immunocompromised patients, resulting in respiratory infection [7].

Classically, *C. propinquum* is distinguished from its closest phylogenetic relative *C. pseudodiphtheriticum* with the absence of urease activity[1]. However, a recent report[17] has demonstrated the existence of urease-producing *C. propinquum* strains and taken attention to probability of misidentification of commercial identification panels, where the presence or absence of urea hydrolysis was the key in assigning strains to *C. propinquum* or *C. pseudodiphtheriticum*. In this case, our isolate was urease positive and on the basis of biochemical reactions, particularly urea hydrolysis, the BD Phoenix system misidentified the organism as *Corynebacterium pseudodiphtheriticum*. On the other hand, MALDI-TOF MS identified the organism as *Corynebacterium propinquum* in accordance with the identification given by 16S rDNA sequence analysis, which is the genotypic confirmation method. This is due to its identification principle based on the analysis of mass spectra of protein molecules instead of biochemical reactions of bacteria. Recently, the usefulness of MALDI-TOF MS has been demonstrated for identifying and discriminating between *Corynebacterium* strains[17,18]. Older publications reporting *C. pseudodiphtheriticum* infections based solely on biochemical tests, mainly detection of urea hydrolysis, may in fact have been incorrectly identified, suggesting the true incidence of *C. propinquum* infections might be higher. Definitive identification of these species should include genetics-based identification methods or MALDI-TOF MS, which was proven to be a rapid, cost-effective replacement for phenotype-based identification methods[17-19].

The organism was susceptible to most of the

antibiotics tested including vancomycin but resistant to clindamycin. Although *C. propinquum* is generally susceptible to vancomycin, the empirical drug of corynebacterial infections[20], resistance to this antibiotic has been reported in a multidrug-resistant strain[11]. Thus, antibiotic sensitivity testing is recommended for prescribing the appropriate treatment.

In conclusion, isolation of a coryneform bacterium from a respiratory specimen has to be critically evaluated and followed by an accurate identification of the strain, ideally beyond the classical methods. Gram staining of the specimen is an important diagnostic tool to assess clinical relevance of these bacteria. *C. propinquum* must be considered as a possible causative agent of respiratory infections, particularly in patients with pre-existing pulmonary diseases or immunosuppressive conditions. Antimicrobial susceptibility testing of *C. propinquum* is important for directing the therapy of infected patients.

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