PLEURAL EFFUSION DUE TO *CORYNEBACTERIUM PROPINQUUM* IN A PATIENT WITH SQUAMOUS CELL CARCINOMA

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Corynebacterium propinquum (*C. propinquum)* is part of the normal oropharyngeal flora. Originally called CDC coryneform ANF-3 (absolute nonfermenter), it was Riegel et al. in 1993 who proposed the name *C. propinquum.*[1] On gram stain, it shows corynebacterial forms after 24 hours' incubation on sheep blood agar. Colonies appear whitish, nonhemolytic and 1-2 mm in diameter with a matted surface. *C. propinquum* is nonlipophilic, catalase positive, reduces nitrate and hydrolyzes tyrosine, but does not hydrolyze urea or esculin, and also does not ferment sugars. CAMP test for the organism is usually negative.[2] Clinical infections by *C. propinquum* are rare. There has only been one previously reported case of native valve endocarditis due to Corynebacterium ANF-3, in 1994,[3] but there have been no reports of this organism as a causative agent of lower respiratory tract infection in the English and non-English literature over the last 20 years. In this report, we describe a case of pleural effusion which grew *C. propinquum* in a patient with squamous cell carcinoma of the lung. The organism was multiresistant to penicillin, cefuroxime, gentamicin, erythromycin, clindamycin, rifampicin and vancomycin, but sensitive to ceftriaxone, ciprofloxacin, imipenem, tetracycline, and sulfamethoxazole-trimethoprim. To our knowledge, this is the second reported case of clinically significant *C. propinquum* infection.

Case Report

A 70-year-old Saudi male was admitted to King Khalid University Hospital, Riyadh, in August 2000, with complaints of cough, shortness of breath, right-sided chest and abdominal pain of one year's duration. The patient had a history of fever, weight loss, hemoptysis and cough, for which he had been seen at different clinics with no definitive diagnosis made. He had no history of previous admission to hospital or treatment with antibiotics. On admission, he looked pale, cachectic, and had finger clubbing. His temperature was 38°C. Chest auscultation revealed right basal crepitation, and he also had tenderness in the right hypochondrium. Blood investigation showed a leukocyte count of 21.70 x 10^9/L with 91% neutrophils, hemoglobin of 8.4 g/L and ESR of 113 mm/hour. Chest x-ray showed right pleural effusion. Blood culture was taken, and 400 mL of pus was aspirated from the pleural effusion, which showed a leukocyte count of >200,000/mm^3, with 100% polymorphs, and red blood cells of 160/mm^3. Gram stain showed gram-positive coryneform-like bacilli. Ziehl-Neelsen staining for acid-fast bacilli was negative.

The patient was started on ceftriaxone 2 g intravenously per day and 500 mg metronidazole 8 hourly. On day four, ultrasound of the liver and lung revealed thick wall pleural effusion and a collection in the liver, with a possible diagnosis of liver abscess. Brain CT scan showed two-ring lesions, which could either have been brain abscess or metastasis. An intercostal chest tube was inserted for the empyema, and drained 250-300 mL of serosanguineous fluid/day. The pus from both liver and from pleural effusion were negative by routine culture and also for *Mycobacterium tuberculosis.* The patient was continued on ceftriaxone and metronidazole for four weeks. During this period the patient's condition was stabilized and the chest tube was subsequently removed, as there was no more fluid coming out. At a further evaluation of the patient's condition, a liver biopsy was done under CT guide, and histopathology result confirmed a squamous cell carcinoma of possible lung origin, with metastasis to the liver and brain. After consultation with the oncology team, a decision was made to put the patient on palliative treatment, as this type of carcinoma is usually resistant to chemotherapy. The patient died after 46 days of admission.

The pleural fluid was cultured on sheep blood and McConkey agar plates and incubated for 24-48 hours aerobically and anaerobically for the blood agar and only aerobically for 24 hours for the McConkey agar. Antimicrobial susceptibility was tested by Stoke's method and *E*-test (AB Biodisk, Solna, Sweden) on Mueller Hinton agar with 5% sheep blood (Mueller Hinton II, Becton Dickinson, USA). A heavy pure growth of tiny colonies appeared on the sheep blood agar after 24 hours incubation in air which, after a further 24 hours incubation, grew colonies which were ~1 mm in diameter, convex, whitish

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TABLE 1. *MIC results for* C. propinquum.

Antimicrobial agents	MIC result (μg/mL)
Penicillin/ampicillin	1.5
Cefuroxime	8
Ceftriaxone	0.47
Erythromycin	6
Clindamycin	12
Gentamicin	6
Ciprofloxacin	0.006
Rifampicin	3
Vancomycin	64
Imipenem	0.015
Tetracycline	0.75
Sulfamethoxazole/Trimethoprim	2/38

MIC=minimum inhibitory concentration.

nonpigmented and nonhemolytic. Gram stain showed gram-positive club shaped and arranged as coryneform bacteria. The organism was not acid or partially acid fast. There was no growth on the McConkey and the anaerobic agar plates. The isolate was catalase positive, reduced nitrate, and did not hydrolyze urea. Identification was made by the API Coryne System (Bio-Merieux SA, France), suggesting the coryne group ANF. These include positive reaction to pyrazinamidase, negative tests for alkaline phosphatase and pyrrolidonyl arylamidase. Negative tests were also given for sugar fermentation, β-glucuronidase, β-glactosidase, α-glucosidase, N-acetyl-β-glucosaminidase, gelatin hydrolysis and esculin. The organism showed similar susceptibility pattern by both disk diffusion and *E*-test methods. It was susceptible to ceftriaxone, imipenem, ciprofloxacin, tetracycline and sulfomethoxazole-trimethoprim. Table 1 shows the minimum inhibitory concentration (MIC) test results for the antimicrobial agents used.

Discussion

Nondiphtherial coryneform bacteria with pathogenic potential are being increasingly isolated from patients who are immunocompromised or implanted with prostheses.[4] The clinical significance of *C. propinquum* in our patient was based on positive direct gram stain with strong leukocyte reaction and heavy pure isolation from a sterile site.[2] Rapidly growing *Mycobacterium*, *Rhodoccus* spp. or *Nocardia* spp. were among the differential identification of the organism. Identification was made by a combination of morphological characteristics of gram stain, negative acid-fast and partial acid-fast staining of the isolate, biochemical reaction and API Coryne System. The API Coryne System is a reliable and improved system for the identification of most gram-positive rods.[5,6]

C. propinquum is distinguished from a phylogenetically related *C. pseudodiphtheriticum*, which is urease positive, and from the CDC group ANF-1, which is negative for nitrate reduction.[2,7] *C. propinquum* (CDC group ANF-3), previously isolated from native valve endocarditis, was sensitive to most antibiotics and vancomycin.[3] Due to the lack of established standards for coryneform bacteria and a referral sensitivity strain, it is recommended to report MIC results without interpretative criteria.[7] Our MIC was compared with MIC of the isolate from the case of endocarditis and those of *C. pseudodiphtheriticum*.[2,3] Both were sensitive to many antibiotics, including β-lactam antibiotics, aminoglycosides and vancomycin. Multiple antimicrobial resistance has also been reported in *C. jeikeium*, *C. urealyticum*, *C. xerosis*, *C. minutissimum*, CDC coryneform group G and *C. amycolatum*.[7-9] However, these bacteria were in all cases susceptible to vancomycin, the recommended drug for infections caused by these bacteria.[7-9]

Infections due to nonenterococcal vancomycin-resistant gram-positive bacteria are thought to be associated with a mortality rate of 5%-20%, which is similar to infections due to susceptible staphylococci or streptococci.[10,11]

Although there was no history of previous hospital admission or treatment with broad-spectrum antibiotic or vancomycin in our patient, and because the isolate from the case of native valve endocarditis due to *C. propinquum* was sensitive to vancomycin, the origin of vancomycin resistance in our isolate is difficult to explain, but we would like to speculate that its source was probably the upper respiratory tract.

In conclusion, as with other emerging coryneform bacteria, full identification of the isolates, especially when they appear on original plate as pure or predominant, should be performed.[2] In diagnostic laboratories, identification can simply be achieved by biochemical test and API Coryne system.[2,9] Due to the unpredictable susceptibility to antibiotics, susceptibility testing should be performed on all antibiotics including vancomycin.[7] Treatment of vancomycin-resistant infections can be by the third-generation cephalosporins, ciprofloxacin or imipenem.[12] Stringent infection control precautions and prudent use of broad-spectrum antibiotics and vancomycin are essential for the prevention or spread of vancomycin resistance.[13]

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References

1. Riegel P, De Briel D, Prevost G, Jehl F, Monteil H. Proposal of *Corynebacterium propinquum* sp. for Corynebacterium group ANF-3 strains. FEMS Microbiol 1993;113:229-34.
2. Funke G, Von Graevenitz A, Clarridge JE III, Bernard KA. Clinical microbiology of coryneform bacteria. Clin Microbiol Rev 1997;10: 125-59.
3. Petit PLC, Bok JW, Thompson J, Buiting AGM, Coyle MB. Native-valve endocarditis due to CDC Coryneform group ANF-3: report of a case and review of corynebacterial endocarditis. Clin Infect Dis 1994;19:897-901.

4. Sewell DL, Coyle MB, Funke G. Prosthetic valve endocarditis caused by *Corynebacterium afermentans* subsp. lipophilum (CDC coryneform group ANF-1). J Clin Microbiol 1995;33:759-61.
5. Martinez-Martinez L, Suarez AI, Winstanley J, Ortega MC, Bernard K. Phenotypic characteristics of 31 strains of *Corynebacterium striatum* isolated from clinical samples. J Clin Microbiol 1995;33: 2458-61.
6. Freney J, Duperron MT, Courtier C, Hansen W, Allard F, Boeufgras JM, et al. Evaluation of API Coryne in comparison with conventional methods for identifying coryneform bacteria. J Clin Microbiol 1991; 29:38-41.
7. Martinez-Martinez L. Clinical significance of newly recognized coryneform bacteria. Rev Med Microbiol 1998;9:55-68.
8. Soriano F, Zapardiel J, Nietro E. Antimicrobial susceptibilities of *Corynebacterium* species and other nonspore-forming gram-positive bacilli to 18 antimicrobial agents. Antimicrob Agents Chemother 1995;39:208-14.
9. unke G, Punter V, Von Graevenitz A. Antimicrobial susceptibility patterns of some recently established coryneform bacteria. Antimicrob Agents Chemother 1996;40:2874-8.
10. Krcmery V Jr, Spanik S, Trupl J. First report of vancomycin-resistant *Streptococcus mitis* bacteremia in a patient with acute leukemia after prophylaxis with quinolones and during treatment with vancomycin. J Chemother 1996;84:325-6.
11. Krcmery V Jr, Trupl J, Drgona L, Lacka J, Kukuekova E, Oravcova E. Nosocomial bacteremia due to vancomycin-resistant *Staphylococcus epidermidis* in four patients with cancer, neutropenia and previous treatment with vancomycin. Eur J Clin Microbiol Infect Dis 1996;10:233-8.
12. Barnass S, Holland K, Tabaqchali S. Vancomycin-resistant *Corynebacterium* species causing prosthetic valve endocarditis successfully treated with imipenem and ciprofloxacin. J Infect 1991; 22:161-9.
13. Krcmery V Jr, Sefton A. Vancomycin resistance in gram-positive bacteria other than *Enterococcus* spp. Intern J Antimicrob Agents 2000;14:99-105.