

EXIT-SITE INFECTIONS BY NON-DIPHThERIA CORYNEBACTERIA IN CAPD

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Non-diphtheria corynebacteria species cause disease in risk populations such as immunocompromised patients and patients with indwelling medical devices. Despite reports of exit-site infection and peritonitis caused by non-diphtheria corynebacteria, these organisms are frequently dismissed as contaminants. During a 10-year observation period, we prospectively identified 8 cases of exit-site/tunnel infections caused by 2 different species of corynebacteria (*Corynebacterium striatum* in 5 and *C. jeikeium* in 3 cases). Four patients experienced a second episode of exit-site infection 3 months (2 cases), 25 months, and 40 months, respectively, after termination of an oral cephalosporin therapy of 4 to 6 weeks' duration. Non-diphtheria corynebacteria accounted for 9% of all exit-site infections during the study period. All catheter-related infections healed; no catheter had to be removed. The diagnosis of catheter-related non-diphtheria corynebacteria infection may be suspected when Gram stain shows gram-positive rods and with colony morphology and commercial biochemical identification systems. Susceptibility of non-diphtheria corynebacteria to antibiotics may vary, especially in *C. jeikeium*. Virtually all *Corynebacterium* species are sensitive to vancomycin. Empirical antibiotic therapy with vancomycin should be initiated while antibiotic susceptibility testing is being carried out. Oral cephalosporin may be an alternative treatment regimen for exit-site infections if sensitive. This study highlights the importance of non-diphtheria corynebacteria as emerging nosocomial pathogens in the population of end-stage renal disease patients on continuous ambulatory peritoneal dialysis.

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Ever since the introduction of continuous ambulatory peritoneal dialysis (CAPD) in the treatment of end-stage renal disease (ESRD), access-related infections have accounted for considerable morbidity. Despite numerous improvements in the design of the

CAPD technique, patient selection, patient education, and mupirocin administration, catheter exit-site infections (ESIs) and tunnel infections (TIs) still occur. They are reasons for repeated and prolonged antibiotic therapy, recurrent peritonitis, catheter failure, and permanent transfer of the patient to hemodialysis. The majority of ESIs originate with gram-positive bacteria, and *Staphylococcus aureus* and *Staphylococcus epidermidis* are the organisms most frequently recovered from exit sites. Gram-negative ESIs by *Pseudomonas aeruginosa* and *Enterobacteriaceae* are hard to eradicate and may require long-standing treatment with multiple antimicrobial drugs. The longevity of a peritoneal dialysis (PD) catheter depends very much on early diagnosis of the infection, isolation of the causative organisms on culture, and determination of their antimicrobial sensitivity (1–6). In clinical practice, however, culture results may remain negative in 5% – 25% of ESI (2,4,6,7); undefined gram-positive rods are isolated in 5% – 10% (1,8).

The genus *Corynebacterium* consists of a large group of aerobically growing, asporogenous, irregularly shaped gram-positive rods. The primary pathogen is *C. diphtheriae*, which has been studied extensively and is well characterized. Non-diphtheria corynebacteria, major components of the normal flora of human skin and mucous membranes, are commonly isolated from clinical specimens from various sites (9–12). Identification of non-diphtheria bacteria to the species level often causes problems. Even when sent to a reference laboratory, 30% – 50% of coryneform bacteria isolates cannot be reliably identified to the species level (12,13). Consequently, there is a low rate of identification from clinical isolates. Non-diphtheria corynebacteria, originally thought to be mainly contaminants, have been recognized recently as pathogenic, especially in immunocompromised hosts or patients with indwelling catheters. While cases of diphtheria have been decreasing over recent decades, recognized cases of severe infections with non-diphtheria corynebacteria have become more prevalent.

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While sporadic cases of peritonitis by identified corynebacteria (14–18) have been reported, only an anecdotal case of an ESI caused by *C. striatum* was found in the literature (19).

We report here the diagnosis, treatment, and outcome of 12 PD ESIs caused by different non-diphtheria corynebacteria species in 8 ESRD patients treated with CAPD.

PATIENTS AND METHODS

Episodes of catheter-related infection were prospectively studied in 67 consecutive CAPD patients between January 1994 and December 2003. No patient was excluded from the analysis. Follow-up of the patient group represented a total of 292 patient-dialysis-years of observation. All peritoneal access devices were double-cuffed Toronto Western Hospital catheters, using an open surgical implantation technique. Disconnect systems with flush and drain before fill (Baxter Healthcare, Deerfield, Illinois, USA, and Fresenius Medical Care, Bad Homburg, Germany) were used in all patients. Patient training and regular follow-up were provided by a dedicated PD service staff. After initiation of CAPD, patients were evaluated on a monthly basis in the PD outpatient clinic. Routine surveillance and treatment of *S. aureus* nasal carriage was not performed during the study period.

DEFINITION OF INFECTIONS COMPLICATING CAPD

Exit-site infections were diagnosed when there was purulent/and or bloody drainage from the exit site, associated with erythema, tenderness, exuberant granulation tissue, and edema. The extension of the erythema needed to be more than twice the catheter diameter. An acute catheter infection could be accompanied by pain and the presence of scab, but crusting alone was not indicative of infection. Tunnel infections were clinically defined by erythema, edema, and/or tenderness over the subcutaneous pathway, and were further characterized by intermittent purulent, bloody, or gooey drainage that discharged spontaneously or after pressure on the cuff. Tunnel involvement was sonographically diagnosed (using a 7-MHz linear transducer) if there was an area of hypoechogenicity (fluid collection) between the cuff and the surrounding tissues. Catheter TIs were defined as “deep” if involvement of the cuff was diagnosed, or as “superficial” if no involvement of the cuff was detected (8). No area of hypoechogenicity without clinical signs of catheter-related infection was observed. Chronic ESI was characterized by the presence of exuberant granulation tissue, both externally and in the sinus, and symptoms of acute ESI.

We used a practical definition of bacterial CAPD peritonitis that required the presence of two of the following criteria in any combination: (1) presence of organisms on Gram stain or culture of PD fluid; (2) cloudy fluid, with > 100 cells/mm³ and with predominantly (more than 50%) polymorphonuclear cells; and (3) symptoms of peritoneal inflammation (3,5,6). The concepts of relapse, recurrence, and reinfection were also defined in the CAPD population. Relapsing ESIs were arbitrarily defined as another episode of ESI caused by the same genus/species that caused the immediately preceding episode of ESI, and that occurred within 4 – 6 weeks after completion of antibiotic therapy. Reinfection was defined as a new catheter-related episode with the same or a different organism.

LABORATORY DIAGNOSIS OF NON-DIPHThERIA CORYNEBACTERIA

Culture swabs were carefully taken from the depth of the exit site, without touching adjoining skin.

Microscopy: Gram stain showed club-shaped uneven-staining gram-positive rods — single cells often gave V and Z forms; palisades or clustering was also observed. Numerous leukocytes were seen in all stains.

Culture Conditions: The patient's material was cultured on 5% sheep blood agar and chocolate agar for 24 hours at 37°C in a 5% CO₂-enriched atmosphere and on MacConkey agar at 37°C in ambient air.

Colony Morphology: Size, pigment, and hemolysis of the colonies were recorded. On blood agar, colonies of *C. striatum* were moist, white, and smooth, and those of *C. jeikeium* were gray and non-hemolytic. *Corynebacterium xerosis* formed yellowish, dry, granular colonies. Colonies of *C. propinquum* were non-hemolytic, 1 – 2 mm in diameter after 24 hours of incubation on sheep blood agar, and had a matte surface. Colonies of *C. auris* were non-hemolytic, dry, and slightly adherent to the agar, becoming slightly yellowish with time.

Biochemical Identification: Lipophilicity was tested by comparing growth on sheep blood agar and the same supplemented with 0.1% – 1.0% Tween 80, with lipophilic corynebacteria exhibiting colonies up to 2 mm in diameter after 24 hours of incubation on Tween-supplemented plates only. The commercial API Coryne system (bioMérieux, Marcy-l'Etoile, France) in conjunction with the API Coryne database was used according to the manufacturer's instructions except that the strips were incubated not for 24 but up to 48 hours.

Susceptibility Testing: Antimicrobial susceptibility patterns were determined by the agar diffusion method according to National Committee for Clinical

Laboratory Standards. Identification of corynebacteria to the species level was performed by the Max Von Pettenkofer Institute, Department of Medical Microbiology of the University of Munich (Munich, Germany), or by the Department of Medical Microbiology, Dr. Römmler and Colleagues Laboratories (Munich, Germany).

RESULTS

During the study period, 133 episodes of bacterial ESI/TI (1/26 patient-months) and 31 episodes of bacterial peritonitis (1/113 patient-months) occurred in 67 patients. Non-diphtheria corynebacteria species caused 12 ESIs or ESI/TI in 8 patients (9% of episodes). The demographic and renal characteristics of these patients with clinically apparent acute ESI (in combination with superficial TI diagnosed by ultrasound in 5 patients) are given in Table 1.

All patients had been on CAPD treatment for at least 9 months. The exit site appeared normal during all visits. The oldest patient was 58 years. None of the patients had malignancy, chronic infection or inflammatory disease, neutropenia, chronic hepatitis or HIV infection, or prosthetic devices (heart valves, neurological shunts, pace makers, or intravenous catheters) other than the PD catheter. None had a trauma to the exit site, or took a course of antibiotics 3 months prior to the first episode, or was on immunosuppressive drugs. Adequacy of CAPD was maintained in all patients.

Gram stain was reported as showing numerous leukocytes and gram-positive rods in isolates from all patients with acute peritoneal catheter ESI. Cultures revealed pure, heavy growth of *Corynebacterium* species. The API Coryne system identified these corynebacteria as *C. striatum* in 5 cases (combined with

C. auris in 1 patient) and *C. jeikeium* in 3 patients (see Tables 2 and 3).

Based on the results of the Gram stain, oral cephalosporin therapy (cephalexin at a loading dose of 2 g and a maintenance dose of 500 mg every 12 hours, or cefuroxime sodium at 2.25 g and 0.75 g every 12 hours) was started. Once culture results and antimicrobial susceptibility of corynebacteria species were available, cephalosporin therapy was continued over 4 weeks in cases of ESI and 6 weeks in cases of combined ESI and early superficial TI. Local care was intensified. Hypertonic saline exit-site compresses were changed 2 – 3 times per day; soaking solutions such as diluted hydrogen peroxide were administered topically. Cauterization of exuberant granulation tissue in the sinus using silver nitrate was necessary in some cases.

Within 1 week of treatment, there was a favorable response of all catheter-related infections. All non-diphtheria bacteria-caused ESIs resolved within 2 (25%) to 3 weeks (75%). The exit site demonstrated normal appearance after 4 weeks. Combined ESI/TI healed within 3 – 4 weeks after the first dose of antimicrobial therapy. Ultrasound examinations demonstrated no fluid collection. Two cultures taken from healed exit sites at months 1 and 2 after oral antibiotics demonstrated no bacterial growth. However, 4 patients experienced a second ESI with corynebacteria at 3 (2 cases), 25, and 40 months later, respectively. Differentiation of cultures with heavy growth of a single type of *Corynebacterium* revealed *C. striatum* in 2 patients and *C. xerosis* and *C. propinquum* in 1 patient each. Only 1 patient experienced a second ESI with the same *Corynebacterium* sp (*C. striatum*). A second 4-week course of cephalosporin therapy and intensified local care was associated once more with a favorable outcome of the infected exit site.

DISCUSSION

Corynebacteria species rarely cause severe disease in previously healthy subjects but have been recognized more recently as important pathogens in immunocompromised patients, in patients with indwelling medical devices, and after invasive procedures. End-stage renal disease patients have a higher risk for access-related infections by non-diphtheria corynebacteria, in particular ESI/TI (19) and peritonitis (14), and nosocomial endocarditis associated with indwelling intravascular devices (20).

Non-diphtheria corynebacteria are commonly isolated from infected sites in CAPD patients (8,14), but frequently they cannot be identified at the species level and are therefore dismissed as contaminants. Compared to the more common causative organisms

TABLE 1
Characteristics of CAPD Patients with *Corynebacterium* sp-Induced Exit-Site Infection (ESI) and Tunnel Infection (TI)

| | |
|--------------------------------------|--------------|
| Patients [n (M/F)] | 8 (3/5) |
| Age | 42±11 years |
| Renal disease | |
| Chronic glomerulonephritis | 5 |
| Diabetes mellitus | 2 |
| Chronic tubulointerstitial nephritis | 1 |
| Duration on CAPD | 23±16 months |
| Kt/V weekly | 2.2±0.2 |
| Creatinine clearance | 69±5 L/week |
| ESI | 8 |
| Combined ESI and TI | 5 |

Numbers are given as absolute number or mean±standard deviation.

TABLE 2
Biochemical Characteristics of *Corynebacterium spp*

| Taxon | Lipophilicity | Nitrate reduction | Pyrazinamidase | Alkaline phosphatase | Acid production from | | | |
|----------------------|---------------|-------------------|----------------|----------------------|----------------------|---------|---------|----------|
| | | | | | Glucose | Maltose | Sucrose | Mannitol |
| <i>C. striatum</i> | - | + | + | + | + | - | V | - |
| <i>C. jeikeium</i> | + | - | + | + | + | V | - | - |
| <i>C. xerosis</i> | - | V | + | + | + | + | + | - |
| <i>C. propinquum</i> | - | + | V | V | - | - | - | - |
| <i>C. auris</i> | - | - | + | + | - | - | - | - |

+ = positive; - = negative; V = variable.

TABLE 3
Antibiotic Sensitivity Testing of *Corynebacterium spp*

| Case | Organism | Infection | Antibiotic sensitivity | | Treatment regimen | Outcome |
|------|-----------------------------|-----------|-------------------------------|-------------|-------------------|--|
| | | | Sensitive | Resistant | | |
| 1 | <i>C. jeikeium</i> | ESI | Pen, Ceph, Vanc | Gent, Cipro | Cephalexin | 2nd episode <i>C. propinquum</i> 40 months later |
| 2 | <i>C. jeikeium</i> | ESI | Pen, Ceph, Gent, Vanco, Cipro | Ceftazidime | Cefuroxime | Cured |
| 3 | <i>C. jeikeium</i> | ESI/TI | Ceph, Gent, Vanco | Pen, Cipro | Cefuroxime | 2nd episode <i>C. striatum</i> 25 months later |
| 4 | <i>C. striatum</i> | ESI/TI | Pen, Ceph, Vanco | Cipro | Cefuroxime | 2nd episode <i>C. striatum</i> 3 months later |
| 5 | <i>C. striatum</i> | ESI | Pen, Ceph, Vanco, Gent | Cipro | Cephalexin | Cured |
| 6 | <i>C. striatum C. auris</i> | ESI/TI | Pen, Ceph, Vanco, Gent | Cipro | Cephalexin | Cured |
| 7 | <i>C. striatum</i> | ESI/TI | Pen, Ceph, Gent, Vanco, Cipro | | Cephalexin | Cured |
| 8 | <i>C. striatum</i> | ESI/TI | Pen, Ceph, Vanco | Gent, Cipro | Cephalexin | 2nd episode <i>C. xerosis</i> 3 months later |

ESI = exit-site infection; Pen = penicillin; Ceph = cephalosporin (cephalothin-cephalexin, cefoxitin, cefamandole-cefuroxime, cefotaxime, ceftazidime); Vanco = vancomycin; Gent = gentamicin; Cipro = ciprofloxacin; TI = tunnel infection.

of peritoneal catheter-related infections, corynebacteria species are rarely reported in ESI/TI. We have described 8 first episodes of ESI/TI caused by non-diphtheria corynebacteria from a single center, accounting for 6% of cases over a 10-year period. Comparable numbers of ESI/TI by corynebacteria without species identification have been reported by another group (8).

Corynebacteria may cause peritoneal catheter infections by themselves or in combination with other *Corynebacterium* species or bacteria (8). Using colony morphology and commercial identification systems in a routine laboratory setting, the cultured organisms were identified as *C. striatum* in 5 cases and *C. jeikeium* in 3. These *Corynebacterium* species are well-described pathogens in humans. *Corynebacterium striatum* has been shown to cause infections of indwelling foreign material, chronic wound infection, endocarditis, pulmonary infections, meningitis, and osteomyelitis (11,12). *Corynebacterium jeikeium* has been claimed to be the most common cause of diphtheroid prosthetic valve endocarditis (12). Clinical infections with *C. jeikeium* also include septicemia,

pneumonia, meningitis, soft tissue infections, and arthritis (11,12). Clinical signs of peritoneal catheter ESI caused by *Corynebacterium spp* did not differ from other bacterial infections. The isolation of corynebacteria species in pure culture, combined with the presence of clinically apparent acute infection and the favorable response to specific antibiotic treatment, strongly suggested a cause-effect relationship in our patients.

Unfortunately, data on the optimal choice, length, and route of administration of antibiotic therapy for bacterial ESI and TI are limited and depend on the organisms isolated. Ninety percent of *S. epidermidis* and approximately 50% of *S. aureus* or *Pseudomonas aeruginosa* catheter infections resolve with an initial course of 2 - 4 weeks of specific antibiotic therapy (5). The treatment algorithm offered by the International Society for Peritoneal Dialysis states that therapy should be based on the Gram stain, and that an oral cephalosporin is the first-line treatment for gram-positive organisms. The use of vancomycin as initial empirical drug therapy for gram-positive ESI is ordinarily avoided in view of the emergence of vancomycin-

resistant enterococci (5). Accordingly, we used a first- or second-generation cephalosporin and the patients were continued on these antibiotics for 4 (ESI) or 6 (TI) weeks. There was objective improvement of the local aspect of the infected exit site, such as reduction of redness and tenderness, within 7 days following the first dose of cephalosporin. No discharge could be expressed from the exit sinus. Fourteen days to 3 weeks after the start of antibiotic therapy, the exit site had healed completely, and 4 weeks after the first dose of cephalosporin no fluid could be detected within the tunnel by ultrasonography. The complete resolution of all ESIs/TIs, the ability to conserve the catheter, and the absence of clinical or microbiological relapse are all evidence for the success of the therapeutic approach with cephalosporins.

Antibiotic susceptibility of *Corynebacterium spp* can vary, however, and susceptibility testing is recommended to determine the best treatment (11). A review by Riegel *et al.* (21) on identification and antimicrobial sensitivity of 415 corynebacterial isolates from clinical specimens of hospitalized patients demonstrated that many species were susceptible to ampicillin, cefotaxime, and vancomycin. Of importance, *C. jeikeium* was resistant to nearly every antibiotic tested. In particular, patients with prolonged hospitalization, neutropenia, or on a prolonged course of antibiotics may have a high prevalence of highly resistant JK corynebacteria (21–23). The drug of choice against non-diphtheria corynebacteria may be vancomycin. In *in vitro* testing, virtually all species of corynebacteria organisms are susceptible to this agent; it is the most reliable antibiotic to use while awaiting susceptibility testing results. Vancomycin therapy was successful in a case of ESI by *C. striatum* (19), but short-term vancomycin-based antibiotic therapy of CAPD peritonitis caused by different species did not prevent relapses (24,25).

During the follow-up period, 4 of the 8 patients with ESI/TI experienced a second catheter-related infection caused by these organisms. In 2 cases with previous infection by *C. jeikeium*, acute ESI occurred 25 and 40 months, respectively, after the first episode. Cultures showed pure growth of *C. striatum* and *C. propinquum* respectively. The other 2 patients had access infections by *C. striatum* and both presented after 3 months with an acute ESI caused by either *C. striatum* or *C. xerosis*. They all were successfully treated with a second course of cephalosporin. Nevertheless, the question remains whether these patients had relapsing infection, recurrent infection, or reinfection with a different corynebacteria species. Several arguments favor our notion that the second episode of *Corynebacterium spp*-caused access infection was not relapsing infection but rather reinfection or recurrent infection. This belief is supported

by the long interval between cessation of antibiotic therapy and recurrence of ESI, the lack of signs of chronic infection during the interval, and two cultures with no microbial growth. However, many of the corynebacteria cannot be specified or typed easily using the commercial API Coryne system in conjunction with the API Coryne database, even in experienced clinical laboratories. In fact, strains of *C. xerosis* and *C. striatum* have been misidentified, and strains of *C. minutissimum* were falsely identified as *C. jeikeium* (11). Recent advances in polymerase chain reaction technology have improved the ability to identify these bacteria and have more clearly defined the microbiological classification of these organisms (26). Powerful methods such as ribosomal sequence analysis are restricted to research laboratories and are not available for the clinical care of PD patients.

In summary, the cases reported highlight the importance of non-diphtheria corynebacteria as nosocomial pathogens in the ESRD population treated with CAPD. These organisms have been underreported, but they may account for at least 5% of cases of ESI/TI. The recognition of infections caused by non-diphtheria corynebacteria is, however, highly dependent on the laboratory's ability to identify these organisms to the species level. Many non-diphtheria corynebacteria-caused access infections are treated effectively by antibiotics and local care. Susceptibility testing of corynebacteria is essential.

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