Isolation of *Corynebacterium* Group D2 from Two Dogs with Urinary Tract Infections

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*Corynebacterium* group D2 was isolated from two dogs with urinary tract infections. The isolates were resistant in vitro to all tested antibacterial drugs except vancomycin. One dog was successfully treated with this antibiotic, while the other died before treatment could be initiated.

Coryneform bacteria are gram-positive microorganisms morphologically resembling diphtheroids and traditionally considered saprophages. More recently, however, some species of coryneform bacteria have been recognized as opportunistic human pathogens. One such microorganism, *Corynebacterium* group D2 (not yet recognized as a legitimate species) (5), has been isolated from human cases of urinary tract infections (UTI). The morphological, biochemical, epidemiological, and pathological characteristics of *Corynebacterium* group D2 have been reviewed (3, 10).

Some species of coryneform bacteria, such as *Corynebacterium pseudotuberculosis* or *C. renale*, are commonly associated with animal pathology. Other microorganisms of this group have been isolated from various organs of animals as part of the normal flora, or, more rarely, in concomitance with pathological processes and have usually been identified only as *Corynebacterium* spp. (1, 4, 6–8, 12). To the best of our knowledge, this is the first report of coryneform bacteria in general and of *Corynebacterium* group D2 specifically being involved in canine UTI.

**CASE REPORTS**

**Case 1.** Case 1 was one of a 3-year-old mixed-breed male dog suffering from hematuria and incontinence. These symptoms had appeared 6 weeks earlier following a road traffic accident, as a result of which the seventh lumbar and first sacral vertebrae were fractured. Treatment with norfloxacin and gentamicin during these 6 weeks had led to a brief period of improvement followed by a relapse. No bacteriological examination of the urine was performed during this period. Upon admission to the hospital, the dog was apyretic and had a leukocyte (WBC) count of 37 × 10^9/liter (normal range, 8 × 10^9 to 18 × 10^9/liter), with 84% neutrophils (normal range, 60 to 77%) and a slight left shift. A modest quantity of ammonium magnesium phosphate (struvite) crystals was found in the urine. *Corynebacterium* group D2, in pure and heavy cultures, was the only microorganism isolated from the urine. No colony counting was performed on this sample. During the following 5 months, various treatments were attempted, including acidification of the urine, cystic rines with 1% Formalin, and antibiotic therapy with cephalaxin, gentamicin, and rifampin. Despite these treatments, the WBC count increased up to 108 × 10^9/liter, and there was no decrease in the quantity of struvite crystals found in the urine. Seven more urine samples were bacteriologically examined during this period. *Corynebacterium* group D2 was isolated from the first three, the fifth, the sixth, and the seventh of these samples in pure cultures. A quantitative examination of these six samples resulted in counts of between 2 × 10^6 and 4 × 10^7 *Corynebacterium* group D2 CFU/ml of urine. *Pseudomonas aeruginosa* (1.5 × 10^5 CFU/ml of urine) was isolated in pure cultures from the fourth of these samples. Finally, the dog was treated with 125 mg of vancomycin (10 mg/kg of body weight) suspended in 100 ml of glucose by slow intravenous infusion for 30 min every 12 h for 7 days. Following this treatment, the WBC count decreased to 8.7 × 10^9/liter, only traces of blood remained in the urine, and the incontinence disappeared. A *Proteus* sp. (7 × 10^6 CFU/ml of urine) was isolated in pure cultures from urine sample 3 days after initiation of the treatment, but subsequent samples were sterile. Three more urine samples examined bacteriologically during the following month were negative, and there was no clinical relapse.

**Case 2.** Case 2 was one of a 10-year-old male Doberman pinscher suffering from posterior paresis subsequent to a traumatic injury induced by jumping. In the past, the dog had suffered from episodes of UTI but was successfully treated with antibiotics. A myelographic examination revealed a constriction of the medullary canal between the first four lumbar vertebrae. Feces were passed normally. The bladder was enlarged and contained 1,500 ml of urine, in which blood traces and a moderate amount of struvite crystals were found. Blood parameters were normal, with a WBC count of 16.5 × 10^9/liter and slight neutrophilia (85%). A radiologic examination revealed in the enlarged bladder radiopaque material resembling calculi and one small calculus in the prostatic urethra. *P. aeruginosa* was isolated in pure cultures from the urine. The dog underwent surgery, during which it was neutered and dorsal laminectomy, prostatectomy, and cystotomy were performed. A histopathologic examination of the prostatic gland revealed signs of a chronic inflammatory process and a hypertrophic bladder wall. Four days after the surgical intervention, *P. aeruginosa* was isolated in pure cultures once more. Colony counting was not performed on these two samples. The WBC count increased to 24.2 × 10^9/liter. The dog was treated with norfloxacin and showed clear signs of improvement.

One week after the intervention, a profuse purulent urethral discharge was observed. During the following 2 weeks, the dog’s general condition deteriorated quickly, despite

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cystic rinses with neomycin and treatment with norfloxacin and later with rifampin. The body temperature increased to 39.7°C (normal temperature, 38.5°C), and the WBC count increased to 32 × 10^9/liter. Biochemical examination of the blood revealed 159.1 μmol of creatinine per liter (normal range, 44.3 to 138.4 μmol/liter), 20 mmol of urea per liter (normal range, 3.1 to 9.2 mmol/liter), and 78 g of total protein per liter (normal range, 55.1 to 75.2 g/liter), probably indicating a state of bacteremia and pyelonephritis. Corynebacterium group D2 was isolated in pure cultures from two samples of urine taken during this week. Colony counting performed only on the second sample yielded 4 × 10^9 CFU/ml of urine. The dog died at the end of this week. At the request of the owners, no necropsy was performed, preventing the examination of internal organs and analysis of the calculi.

Attempts to isolate Corynebacterium group D2 from skin swabs were unsuccessful in both cases because of overgrowth of the culture media by saprophages.

**MATERIALS AND METHODS**

Urine was collected in both cases by catheterization with a sterile catheter, which was introduced after preputial disinfection with 0.015% chlorhexidine gluconate (Savior Hospital Concentrate; Abic Ltd., Chemical and Pharmaceutical Industries, Netanya, Israel). Urine samples were inoculated onto 5% sheep blood agar, nutrient agar, and MacConkey agar (Difco Laboratories, Detroit, Mich.) and incubated aerobically at 37°C. Anaerobic culturing was not performed. The isolates were tested with the API-Coryne system (API-bioMérieux, Inc., Marcy-l'Etoile, France) for nitrate reduction; the production of pyrazinamidase, pyrrolidonyl arylamidase, alkaline phosphatase, β-glucuronidase, β-galactosidase, α-glucoamidase, N-acetyl-β-glucosaminidase, esculin (β-glucosidase), and urease; the hydrolysis of gelatin; and the fermentation of glucose, ribose, xylose, mannitol, maltose, lactose, sucrose, and glycogen. In addition, the following were tested: the fermentation of glucose, maltose, sucrose, mannitol, xylose, lactose, ribose, and glycogen (enteric fermentation base with 1% carboxylate); the hydrolysis of nitrate (nitrate broth), hippurate (heart infusion broth with 1% sodium hippurate), esculin (heart infusion agar with ferric citrate and 1% esculin), and gelatin (heart infusion broth with 12% gelatin); and the production of acetoin in the Voges-Proskauer test (peptone with dipotassium phosphate and 1% glucose). In addition, the production of acetoin (Voges-Proskauer test), hippurate hydrolysis, and the production of leucine aminopeptidase and alkaline phosphatase were tested with diagnostic discs (diagnostic tablets for microbial identification; Rosco Diagnostica, Taastrup, Denmark).

Colony counting was performed by inoculating 0.1 ml of 10-fold serial dilutions of urine in 0.9% sterile saline onto 5% sheep agar plates. The results were read after 48 h of incubation at 37°C.

In vitro disc susceptibility tests were performed and interpreted in accordance with National Committee for Clinical Laboratory Standards criteria (9). The antibacterial drugs tested were amikacin, ampicillin, amoxicillin-clavulanic acid, cefonicid, cefotaxime, cephalothin, chloramphenicol, clindamycin, erythromycin, gentamicin, methicillin, neomycin, nitrofurantoin, norfloxacin, penicillin, rifampin, sulfonamides with and without trimethoprim, tetracycline, nalidixic acid, and vancomycin. The influence of serum on the in vitro susceptibilities of the isolates to vancomycin was tested by supplementing Mueller-Hinton agar with 2% horse serum.

The MIC and MBC were determined in nutrient broth with 2% horse serum and twofold increasing dilutions of vancomycin.

**RESULTS AND DISCUSSION**

The morphological and biochemical characteristics of the two isolates as well as their in vitro susceptibilities to the antibacterial drugs tested were identical.

After 48 h, nonhemolytic pinpoint-sized colonies appeared on the blood and nutrient agars. There was no growth on the MacConkey agar. The colonies consisted of gram-positive coryneform microorganisms. They were strictly aerobic and catalase positive. Growth was enhanced by the addition of 2% horse or bovine serum to the culture media.

With the API-Coryne system, pyrazinamidase and urease were positive, the latter almost instantaneously. Urease was produced on Christensen's medium as well. All the other tests were negative. Accordingly, the isolates were identified as *Corynebacterium* group D2.

There was no inhibition zone around any of the antibacterial disks-impregnated discs tested except that impregnated with vancomycin, which was surrounded by an inhibition zone of 28 mm. The addition of 2% horse serum to Mueller-Hinton agar did not modify the diameter of this inhibition zone. The MIC and MBC of vancomycin were 0.75 and 6 μg/ml, respectively.

The two isolates described above did not differ in their biochemical characteristics from human strains and varied only slightly in their in vitro susceptibilities to the antibacterial drugs tested (11). Furthermore, as in human cases (10), *Corynebacterium* group D2 was isolated from animals at risk of UTI and exposed to a nosocomial environment. In addition, bacteremia, pyelonephritis, and the formation of struvite crystals in the urine and probably of calculi in the bladder and the prostatic urethra were associated with the presence of the organism in the urinary tract.

The source of *Corynebacterium* group D2 isolated from the dogs with UTI was probably environmental, possibly (although not demonstrated) from the skin. Preputial disinfection, the purity of the cultures, and the uneventful recovery of the first dog after specific anti-*Corynebacterium* group D2 treatment are likely to have excluded the possibility of contamination with this microorganism during sampling. Although no generally accepted standard for the quantitative bacteriological examination of animal urine exists (2), high colony counts of the infecting microorganism further support this assumption. Consequently, *Corynebacterium* group D2 seems to be an opportunistic pathogen, comparable to the gram-negative microorganisms isolated from the urine of the dogs. It is noteworthy, however, that while antibacterial therapy against *P. aeruginosa* and the *Proteus* sp. led to only a temporary improvement in the condition of the first dog, similar treatment against *Corynebacterium* group D2 seemingly resulted in a complete cure of the UTI.

The classification of coryneform bacterial species has been a relatively complex procedure. With the advent of identification kits, the task has been greatly simplified. Routine species identification of such microorganisms whenever they are involved in animal pathological processes can now be easily performed. The information thus gained could improve the understanding of the role of coryneform bacteria in such processes and eventually clarify the significance.
of species, as in the cases reported here, that have been implicated in both human and animal infections.

**ADDENDUM IN PROOF**


**REFERENCES**