**Abstract**

Moraxella osloensis, a gram-negative bacterium that is saprophytic on skin and mucosa, rarely causes infections. Moreover, infections in patients with cancer have not been reported. We describe 10 cases of M. osloensis blood or catheter infections that occurred during anticancer chemotherapy with or without preexisting neutropenia. The organism was identified definitively by sequencing analysis of the 16S ribosomal RNA gene. Fever (up to 39.7°C) with substantial neutrophilia characterized these infections. The infections were monomicrobial for 3 patients and polymicrobial for 7 patients. Nine patients acquired the infection through central venous catheter colonization. The likely sources of the organism were sinusitis (3 cases), bronchitis (1 case), presumed subclinical mucositis from anticancer therapy (4 cases), and cutaneous graft-vs-host disease (2 cases). The infections resolved, without catheter removal, after antibiotic therapy with cell wall–active agents, to which all strains were shown to be susceptible. The M. osloensis strains exhibited significant morphologic variations on Gram stain, and sheep blood agar was the preferred culture medium for 9 strains.

The gram-negative bacteria Moraxella species (excluding Branhamella [Moraxella] catarrhalis) are environmental and skin and mucosal dwellers and rarely cause human infections. They are asaccharolytic, oxidase-positive, indole-negative, and cocccoid to bacillary shaped. Several Moraxella species, ie, Moraxella atlantae, Moraxella canis, Moraxella lacunata, Moraxella lincolnii, Moraxella nonliquefaciens, and Moraxella osloensis, can be isolated occasionally from clinical specimens. In a study of 933 isolates of Moraxella species and other closely related organisms that were referred to the Centers for Disease Control and Prevention, M. nonliquefaciens was the most frequently isolated species, whereas M. osloensis was associated most frequently with a systemic disease syndrome. Unfortunately, little clinical information on the “systemic disease syndrome” was available owing to the reference nature of the study. Instead, the current medical literature on M. osloensis essentially comes from case reports. Since the description of M. osloensis as a distinct species in 1967, there have been only 16 reported cases to our knowledge. All cases, except the 2-case series of M. osloensis endophthalmitis, were individual case reports of bacteremia, central venous catheter (CVC) infection, meningitis, synovitis, osteomyelitis, peritonitis, and pneumonia. Half of the 16 cases were children. M. osloensis infections in patients with cancer, an enlarging population who are prone to various infections, have not been reported.

Differentiation of M. osloensis from other Moraxella species via biochemical tests is cumbersome. Most clinical microbiology laboratories rarely attempt to do so because of the infrequent isolation of these organisms, uncertain clinical relevance, and possible inaccuracy. However, accurate
identification of *M osloensis* can be achieved through sequence analysis of the 16S ribosomal RNA gene (16S rRNA). By using such sequencing analysis, we identified 10 cases of *M osloensis* bacteremia or catheter infections that occurred during anticancer chemotherapy. The clinical and microbiologic features of these cases are presented, and likely sources of the infections are hypothesized.

**Materials and Methods**

The cases occurred sporadically from January 2002 to June 2003 at the University of Texas M.D. Anderson Cancer Center, Houston, a 500-bed tertiary cancer center. They were identified among the approximately 38,000 blood cultures performed during the 18 months. The bacteria were isolated from blood cultures using the Bactec 9240 automated culturing system (BD Diagnostic Systems, Sparks, MD) and Isolator tubes (Wampole Laboratories, Princeton, NJ). When an Isolator tube culture was positive, the number of bacterial colonies was quantitated from the 10 mL of blood cultured. All subcultures were plated on blood agar and chocolate agar (BBL, Becton Dickinson Microbiology Systems, Cockeysville, MD) and incubated aerobically at 35°C with 5% carbon dioxide. The morphologic features of the colony were observed and size was measured under a dissecting microscope. The clinical information for each patient was obtained through review of the medical records.

The organisms were identified presumptively as *Moraxella* species on the basis of gram-negative stain, slight fastidiousness on culture, positive reaction for oxidase, and lack of reactivity in the majority of routine biochemical tests. The definitive identities were achieved through sequence analysis of a portion of the 16S rDNA described previously. Briefly, genomic DNA from pure culture colonies was extracted and subjected to amplification by a polymerase chain reaction for a 593-base-pair fragment of the 16S rDNA. A set of universal bacterial primers, 5′-TGCCAGCAGCCGCGGTAATAC-3′ and 5′-CGCTCGTGTTCCGGACTTAC-3′ (positions 515-1107 of GenBank accession J01859 of *Escherichia coli*), was used for the amplification. The amplicon was sequenced by the dye-terminator method in an ABI 377 sequencer (Applied Biosystems, Foster City, CA), and sequence analysis was performed through a GenBank BLAST query. The antibiotic susceptibility test was performed using Etest (AB Biodisk, Solna, Sweden) after a 48-hour incubation and interpreted according to the breakpoints set for *Pseudomonas aeruginosa* and non-Enterobacteriaceae by the National Committee for Clinical Laboratory Standards.

**Results**

**Identification and Microbiologic Features**

The 16S rDNA sequences of the bacteria all matched best, from 99% to 100%, with several *M osloensis* sequences in the GenBank, including the type strain NCTC 10465 (X74897), strain 5873 that was used for phylogenetic analysis (AF005190), and a few environmental strains. The matches with other *Moraxella* species were all less than 96%. Therefore, the bacteria were identified definitively as *M osloensis*. It generally is accepted that a 99% match of the 16S rDNA sequences identifies an organism at the species level if the phenotypic features also are compatible.

Significant morphologic variations of these strains were noted on Gram stain. As shown in Image 1, 4 strains (Image 1A, from cases 2-4 and 6) were small bacilli to coccobacilli, the typical morphologic features known for *M osloensis*; 4 strains (Image 1B, from cases 7-10) were cocci to diplococci; and 2 strains were pleomorphic, including cocci, coccobacilli, and large, long bacilli (Image 1C, from cases 1 and 5). The pleomorphic organisms (Image 1C) also were gram-variable, which, on initial examination, mimicked a yeast or a *Bacillus* species. These variations were stable after subcultures and storage at –80°C.

Other phenotypic features were consistent among the strains, most notably the asaccharolytic reactions, slight fastidiousness (48-hour incubation on blood or chocolate agar), small and nonhemolytic colonies on sheep blood agar, and light yellow color on a cotton swab. The fastidiousness of the organism also was reflected by varying growth on sheep blood and chocolate agar: of the 10 strains, 2 showed equal growth on both agars (1 mm at 48 hours), 7 had better growth (0.5-1.2 mm; mean, 0.8 mm at 48 hours), and 1 grew better on chocolate agar (0.6 mm at 48 hours) but poor to variable growth on chocolate agar, and I grew better on chocolate agar (0.6 mm at 48 hours) but barely on blood agar. Overall, blood agar was the preferred medium for all except 1 strain. The colonies were clear within 48 hours and became gray at prolonged incubation. There was no relationship between the requirement of growth medium and the cellular morphologic features on Gram stain.

The *M osloensis* strains were tested against a panel of antibiotics, and all were susceptible to amikacin (minimum inhibitory concentration, 0.25-2.0 µg/mL), cefepime (0.023-0.064 µg/mL), ciprofloxacin (0.064-0.25 µg/mL), imipenem (0.016-4.0 µg/mL), ticarcillin-clavulanate (0.016-0.064 µg/mL), and trimethoprim-sulfamethoxazole (0.064-3.0 µg/mL). They also were negative for β-lactamase through a cefinase test, which is one of the characteristics of *Moraxella* species, ie, being exquisitely susceptible to penicillin.
The clinical features of the patients are summarized in Table 1. There were 5 men and 5 women with an age range of 21 to 65 years (median, 49 years). Of the 10 patients, 6 had a primary diagnosis of lymphoma or leukemia, 2 had a solid tumor, and 2 were stem cell transplant recipients. The treatment modalities varied. Eight patients had the infection during chemotherapy or between cycles and had a fever (38°C to 39.7°C; median, 39°C), whereas the 2 stem cell transplant recipients did not have fever. All patients recovered from the infections without requiring catheter removal. In the absence of apparent catheter site infections, the presumed sources of *M. osloensis* were sinusitis (cases 2, 4, and 5), possible bronchitis (case 6), subclinical mucositis (cases 1, 3, 7, and 8), and cutaneous graft-vs-host disease (GVHD; cases 9 and 10). The infections were monomicrobic in 3 cases and polymicrobic in 7 cases. The cases are presented briefly as follows.

**Monomicrobic Infections**

**Case 1**

The patient had a fever and leukocytosis (1.8-fold rise from baseline). A culture of the peripheral blood sample grew pure *M. osloensis* (positive bottle only without quantitation). A simultaneous culture of the CVC blood sample also grew this organism and 2 others. Thus, catheter-related bloodstream infection was diagnosed according to our institutional practice.\(^{15}\) The patient was treated with intravenous vancomycin; his fever subsided in 5 days, and the leukocytosis subsided in 3 days.

**Case 2**

The patient had a fever, marked leukocytosis (2.5-fold), sinus drainage, and radiologically documented sinusitis (without a culture). A blood sample obtained through the CVC grew pure *M. osloensis*. A simultaneous peripheral blood culture was sterile. After treatment with oral amoxicillin plus clavulanate, her body temperature and WBC count returned to normal (6,800/µL [6.8 × 10⁹/L]) the next day, and the sinusitis resolved 4 days later.

**Case 3**

The patient was admitted to the hospital because of a high fever, chills, and mild hypotension. Pure *M. osloensis* was cultured from the CVC blood sample, but not from the peripheral blood sample. The lower colony count likely was related to the oral levofloxacin prophylaxis for her leukopenia. Despite treatment with intravenous vancomycin and imipenem plus cilastatin, the fever persisted for a week before resolution. Follow-up blood cultures from CVC and peripheral blood samples were negative.

**Polymicrobic Infections**

In addition to *M. osloensis*, the polymicrobial infections also included 1 or 2 of skin or environmental flora: *Micrococcus* species, *Acinetobacter* species, Coryneform bacillus, coagulase-negative *Staphylococcus*, *Brevundimonas diminuta*, and *Brevibacterium casae* (a Coryneform bacillus) (Table 1). Fever (38°C to 39.2°C) was present in all cases except 2.
Case 4

The patient had maxillary sinus pain, a nasal drainage, and radiologic evidence of sinusitis (no culture). He had been taking oral levofloxacin prophylaxis for profound neutropenia (160/µL [0.16 × 10⁹/L]). The patient was treated with oral amoxicillin plus clavulanate for this infection in the clinic. A culture of a CVC blood sample later grew *M. osloensis* and *Micrococcus* species (with a negative peripheral blood culture). However, the sinusitis and fever persisted for 2 days, which prompted an emergency department (ED) visit and hospital admission. Intravenous cefepime was added to the treatment regimen, and the patient recovered 2 days later. Cultures of CVC and peripheral blood samples obtained in the ED were negative.

Case 5

The patient had a possible sinusitis because of sinonasal congestion and headache for several days (without a roentgenogram). The patient had been receiving ciprofloxacin prophylaxis. However, culture of the CVC blood sample grew *M. osloensis* and *Micrococcus* species heavily and *Acinetobacter* species. She was treated with cefepime in the ED and discharged without recurrence. A culture of a peripheral blood sample was sterile.

Case 6

The patient was admitted to the hospital owing to sepsis and severe leukopenia. She also had possible bronchitis (dry cough and shortness of breath). A culture of a CVC blood
# Bacteria Cultured (Colonies/10 mL Blood) | Treatment | Outcome
--- | --- | ---
From CVC: *Moraxella osloensis* (31-50); *Micrococcus* species (51-100); Coryneform bacillus (13-15); from peripheral blood; *M. osloensis* only | Ciprofloxacin, vancomycin | Recovery; catheter remained
From CVC: *M. osloensis* (201-500); from peripheral blood, negative | Amoxicillin plus clavulanate | Recovery; catheter remained
From CVC: *M. osloensis* (8); from peripheral blood, negative | Vancomycin | Recovery; catheter remained
From CVC: *M. osloensis* (1); *Micrococcus* species (13-15); from peripheral blood, negative | Amoxicillin plus clavulanate; cefepime added later | Recovery; catheter remained
From CVC: *M. osloensis* (101-200); *Micrococcus* species (101-200); *Acinetobacter" species (3); from peripheral blood, negative | Cefepime | Recovery; catheter remained
From CVC: *Brevundimonas diminuta* case (1,000); *M. osloensis* (7); *Micrococcus* species (13-15); no peripheral blood sample cultured | Cefepime, imipenem plus cilastatin | Recovery; catheter remained
From CVC: *M. osloensis* (4); *Micrococcus* species (31-50); coagulase-negative *Staphylococcus* (51-100); from peripheral blood, negative | None | Resolved
From CVC: *M. osloensis* (13-15); Coryneform bacillus (101-200); from peripheral blood, negative | Levofloxacin and imipenem | Recovery
From CVC: *M. osloensis* (5); *Bravundimonas diminuta*; no peripheral blood sample cultured | Levofloxacin | Recovery
From CVC: negative; from peripheral blood; *M. osloensis*; *Micrococcus* species | Ampicillin and ceftriaxone | Recovery, catheter remained

The patient visited the ED because of fever, headaches, and vomiting; mild hypotension and neutrophilia were noted. He was treated with oral levofloxacin in the ED and discharged. Blood cultures grew *M. osloensis* and a Coryneform bacillus 3 days later from the CVC sample but not from the peripheral blood sample. Then, the patient’s fever had subsided, but he was still admitted for intravenous imipenem in view of his neutropenia (260/µL [0.26 × 10⁹/L]). The catheter was removed 2 days later owing to completion of chemotherapy, and it was sterile.

Case 10

The patient also was a stem cell transplant recipient with cutaneous GVHD treated with tacrolimus. Although afebrile, he had substantial leukocytosis (1.8-fold from baseline) despite prophylactic use of trimethoprim-sulfamethoxazole. A CVC blood culture grew *M. osloensis* and *B. diminuta* 2 days later. A peripheral blood sample was not obtained. He was treated further with oral levofloxacin.

### Discussion

Our cases (to our knowledge the only case series on systemic *M. osloensis* infections so far and the only study of such infections in patients with cancer) raised a few interesting points regarding the occurrence frequency, clinical and microbiologic features, and source of the infection.

## Occurrence and Microbiologic Features

The occurrence of 10 cases of *M. osloensis* blood or catheter infection within 18 months in our patients suggests...
that this infection is not as rare as initially thought, particularly among patients with cancer who frequently have catheters in place and have anticancer chemotherapy–related mucositis. During the 18 months, significant isolates of Moraxella species other than M osloensis were not noted, suggesting that M osloensis is likely the most clinically significant species in the genus. The isolation frequency was similar to or slightly higher than the occurrence of B catarrhalis during the same period, which was isolated from the airway of 6 patients and the eyes of 1 patient but none from blood (X.Y.H., unpublished data). M osloensis and B catarrhalis belong to the same family, Moraxellaceae, and probably the same broader genus, Moraxella, according to a recent taxonomy; however, at the 16S rDNA level, the 2 organisms match at a 92% level, suggesting substantial phylogenetic distance.

Based on biochemical characterization, M osloensis commonly is considered a gram-negative bacillus, although morphologic variations have been noted. Our strains, being definitively identified by 16S rDNA sequencing, showed that such morphologic variation was common and significant, ranging from small bacillus, cocacobacillus, coccus, and diplococcus to large pleomorphic bacillus. Some strains also appeared gram-variable. The coccal and diplococcal morphologic features might cause confusion with B catarrhalis, while the large pleomorphic bacilli might lead to identification uncertainty at the genus or species level. Such morphologic variation, we speculate, might have contributed to the previous underrecognition of the organism. Except these cellular variations, other phenotypic features were consistent among our M osloensis strains, namely the asaccharolytic reactions, slightly fastidious growth, and light yellow colony color on the cotton swab. The fastidious growth is distinct from that of B catarrhalis and M nonliquefaciens, both of which grow readily after a 24-hour incubation.

Clinical Features

Nine of our 10 patients had significant CVC-related infection, with only 1 patient being profoundly neutropenic (case 4), suggesting considerable pathogenicity of the organism. All patients except the last 2 had fever, the highest being 39.7°C. With the exception of the severely neutropenic patient (case 4), neutrophilia was another consistent feature, accounting for 69% to 97% (0.69-0.97; median, 90% [0.90]) of the total leukocytes. For patients 1, 2, and 9 who were not leukopenic and had reliable baseline leukocyte counts, these cells increased 1.8- to 2.5-fold in response to the infection.

Fortunately, the infections were not protracted and were controlled by host defenses along with appropriate antibiotic treatment. For example, 7 patients (cases 1-5, 7, and 8) had paired cultures of blood samples from the CVC and the periphery. Only 1 pair grew M osloensis, thus representing catheter-related bloodstream infection (case 1), whereas the other 6 pairs grew the organism only from the CVC blood sample, not from the peripheral blood sample, thus representing catheter colonization and infection. With the use of cell wall–active antibiotics to which all M osloensis strains were susceptible, the infections cleared within 1 to 2 days for patients 1, 2, and 5 who also mounted vigorous leukocyte response, and within 4 to 7 days for patients 3, 4, and 6 who were leukopenic. Therefore, while M osloensis is highly pyrogenic, it is controlled easily by administration of antibiotics, therapeutically or prophylactically (cases 3-5), or by a brisk WBC response (cases 2 and 7). The second patient had pure and heavy growth of the organism from the CVC blood sample. None of the infections required catheter removal. Generally, when a catheter-related bloodstream infection is caused by a gram-negative bacillus, catheter removal is recommended. Our data, thus, suggest a possible exception for M osloensis.

Source of the Organism

M osloensis has a wide distribution in nature. In addition to being parasitic on the mucous membranes of humans and other warm-blooded animals, it also has been found in the hospital environment and recently in waste-processing sludge (Y15855 and AB021321), lake sediment (AB074738), and nematodes and slugs, in which it was pathogenic. In our cases, the likely sources of the organism were documented sinusitis (2 cases), presumed sinusitis (1 case), possible bronchitis (1 case), presumed subclinical anticancer chemotherapy–related mucositis (4 cases), and cutaneous GVHD (2 cases). It was unclear, however, how the organism spread from inflamed sinus or mucosa to the catheter, but we speculate a hematogenous route.

Three of the 4 reported cases of M osloensis bacteremia also were remarkable for mucosal compromise, ie, stomatitis, urethritis, and reactive airway disease. The fourth case had a porcine heart valve. In the only reported case of CVC infection, multiple episodes of sinusitis also were the probable source of CVC colonization. This infection was characterized by high fever (40°C) and good response to antibiotic therapy without CVC removal. In a recently published case of M osloensis pneumonia, the patient, a 6-year-old girl, had a cough for 10 days before the acute onset of pulmonary infection, but she was not bacteremic. Finally, 2 surgical patients who received an anesthetic solution that was contaminated with M osloensis exhibited high fever and hypertension (blood pressure, 226/108 mm Hg) within 2 hours of their operations. In summary, M osloensis causes significant, albeit infrequent, human disease. In patients receiving anticancer chemotherapy with or without preexisting neutropenia, it causes intravascular catheter–related infection. High fever...
and leukocytosis characterize the infection. Sinusitis and other mucosal defects are the likely source of the bacterium for blood entry and CVC colonization. Therapy with penicillin or other cell wall–active antibiotics seems effective in clearing the infection without the need for catheter removal.

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References


