

Isolation of *Enterococcus mundtii* from Normally Sterile Body Sites in Two Patients

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***Enterococcus mundtii*, a recently described nonmotile, yellow-pigmented enterococcal species, was isolated from a chronic thigh abscess and from operatively obtained sinus mucosa. It is emphasized that this species may be encountered in clinical specimens and that the correct species identification may be missed when commercially available identification systems are relied on.**

Enterococci are an important group of potentially pathogenic bacteria whose taxonomy has changed considerably in the last few years. To date, 12 enterococcal species have been described, and recently Facklam and Collins (4) have proposed an identification procedure for differentiating the species which is based on conventional biochemical tests. Some of the more recently described species have been isolated only rarely from clinical specimens.

In the present report, we describe two strains of *Enterococcus mundtii*, and this is to our knowledge the first detailed description of their isolation from normally sterile body sites. These strains were originally identified as *Enterococcus faecium* by a commercially available identification system and were also incorrectly identified as *Enterococcus faecalis* by another commercial system.

Case 1. A 44-year-old female had a history of a severe, steroid-dependent rheumatoid arthritis with multiple joint involvements. Therefore, several surgeries were necessary, including a right total hip arthroplasty in 1978.

In March 1985, the patient was admitted again for evaluation of fevers with chills. Laboratory studies showed a leukocytosis of 88,000 cells per mm³, with a shift to immature neutrophils. Despite empirical antibiotic therapy with trimethoprim-sulfamethoxazole plus nafcillin, the patient remained febrile. Several blood cultures were sterile. One week after the hospital admission, an abscess of the right thigh became apparent and a debridement was carried out. *Staphylococcus aureus* and an *Enterococcus* sp. (originally identified as *E. faecium*) were isolated from the intraoperatively obtained tissue. Nafcillin and penicillin were given intravenously for 10 days, and the patient rapidly became afebrile.

In June 1985, the patient became febrile again and underwent removal of the acetabular component of the total hip arthroplasty, with placement of tobramycin beads. Intraoperative cultures again yielded *S. aureus* and an *Enterococcus* species with antibiograms identical to those for the previous isolates. An additional course of trimethoprim-sulfamethoxazole plus cefazolin was given intravenously and was associated with a complete recovery.

Case 2. A 4-year-old boy became ill with chronic granulocytic leukemia in April 1984. After chemotherapeutic immunosuppression, as well as total body irradiation, a first bone marrow transplantation, performed in October 1984, was

complicated by multiple febrile episodes and a lack of engraftment. Despite broad-spectrum antibiotic therapy with various regimens including vancomycin, tobramycin, clindamycin, ticarcillin, cefotaxime, and amphotericin B, the patient remained febrile. Because a chronic sinusitis was considered a possible focus of infection, bilateral nasal antral windows were made. The operatively obtained culture material from the left sinus showed light growth of an alpha-hemolytic oral streptococcus (species identification was not done) and light growth of an *Enterococcus* sp. (originally identified as *E. faecium*), but no anaerobic bacteria were found. The histological examination of the removed sinus mucosa revealed no evidence for a pathological process. During the later clinical course, two further attempts of bone marrow transplantation were unsuccessful, and finally the boy died from progressive encephalopathy and respiratory arrest. At the autopsy, no specific cause of death was identified.

The isolates of both cases were confirmed as enterococci by established routine methods; both strains were catalase negative, they grew in the presence of 6.5% NaCl at 10 and 45°C, and they were positive for pyrrolidonylamidase activity and for Lancefield group D antigen (Phadebact; Pharmacia). The species differentiation with the GPI card (Vitek Systems, Inc., Hazelwood, Mo.) led originally to the identification *E. faecium* with a 99% probability in both cases (code number 77727370160 for case 1 and code number 77727270160 for case 2). With this system, both strains were esculin, lactose, mannitol, trehalose, and arabinose positive; they were raffinose and inulin negative. One strain was sorbitol positive, and the other strain was sorbitol negative.

Later testing with the API 20S Streptococcus System (Analytab Products, Plainview, N.Y.) gave the result *E. faecalis* in both cases (profile number 7702741, "excellent identification" for both). Both strains gave a negative raffinose test; arabinose utilization is not included in the API 20S. Other key reactions included acid formation from mannitol, sorbitol, glycerol, lactose, trehalose, and sucrose. The strains were bile esculin positive, utilized arginine, and were negative for hippurate hydrolysis.

The two isolates were nonhemolytic and yellow pigmented on 5% sheep blood agar plates. Of note, they were nonmotile. Further identification by applying other conventional biochemical tests (Table 1), as described by Facklam and Collins (4), confirmed the final identification of these isolates as *E. mundtii*. Key reactions that facilitated differentiation

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TABLE 1. Results of conventional physiological tests for both *E. mundtii* isolates compared with those for *E. faecalis* and *E. faecium*^a

Test	Result		
	<i>E. mundtii</i> ^b	<i>E. faecalis</i>	<i>E. faecium</i>
Formation of acid from:			
Arabinose	+	— ^c	+ ^c
Sorbitol	+	+ ^c	—
Mannitol	+	+	+
Lactose	+	+ ^c	+
Raffinose	+	— ^c	+ ^c
Trehalose	+	+	+
Inulin	—	—	— ^c
Arginine deamination	+	+ ^c	+
Hippurate hydrolysis	—	Variable	Variable
Voges-Proskauer reaction	+	+	+
Motility	—	—	—

^a All incubations and test interpretations were done as described previously (4).

^b The two strains from our study.

^c Occasional exceptions occur.

from *E. faecalis* and *E. faecium* were arabinose, sorbitol, and raffinose utilization.

The MICs of several relevant antibiotics were determined by a standardized agar dilution method employing Mueller-Hinton agar (7) (Table 2). Testing was not done for trimethoprim-sulfamethoxazole since this drug is not considered effective therapy for enterococcal infections.

Although the strains have been known since 1950 (6), the taxonomy of yellow-pigmented enterococcal strains was equivocal until recently, when they were isolated in greater numbers from soil (9) and plants (10). Some of these strains are motile and belong to the taxon *Enterococcus casseliflavus*, which was proposed in 1984 (2). In 1986, Collins et al. (1) described a new enterococcal species, *E. mundtii*, which encompasses yellow-pigmented nonmotile strains. The division of the yellow-pigmented enterococcal isolates into two distinct groups was supported by their different DNA base compositions. The guanine-plus-cytosine (G+C) content of *E. casseliflavus* ranged from 42 to 45 mol%, in contrast to that of strains belonging to *E. mundtii*, which had a G+C content of 38 to 39 mol%. Furthermore, DNA-DNA hybridization studies showed that strains of *E. mundtii* segregated into a homology group that distinguished them clearly from other enterococcal species.

Two other nonmotile, yellow-pigmented streptococcal

TABLE 2. Antibiotic susceptibility results for both isolates of *E. mundtii*

Antibiotic	MIC (μg/ml) for isolate	
	Case 1	Case 2
Ampicillin	0.5	1
Chloramphenicol	2	4
Ciprofloxacin	1	1
Erythromycin	≤0.5	1
Imipenem	2	2
Tetracycline	≤0.5	≤0.5
Vancomycin	0.5	≤0.5
Gentamicin	16	16
Streptomycin	125	125
Amikacin	125	125

strains are mentioned by Collins et al. (1): one *Streptococcus garvieae* strain, a nonenterococcal species (5), and one enterococcal strain (NCDO 2379, isolated from plants) whose taxonomic position remains unclear.

Since the species description, very few *E. mundtii* strains have been reported, and knowledge about their occurrence in humans (either as commensals or pathogens) is poor. The four strains described by Collins et al. (1) were isolated from cow teats and the hands of milkers and from plants and soil. Devriese et al. (3) recovered three *E. mundtii* strains from the intestines of a cow, a pig, and a horse. The study of selected enterococcal strains conducted by Facklam and Collins (4) included two human *E. mundtii* isolates, although the clinical relevance of these strains was not described.

In a recently published investigation, Ruoff and coworkers (8) applied the same test scheme as Facklam and Collins (4), as well as commercially available identification systems; the study of 302 unselected, consecutive enterococcal isolates from routine cultures revealed no *E. mundtii*. Although the available data indicate that the incidence of *E. mundtii* appears to be low, this enterococcal species may be encountered in clinical specimens.

In one of our reported cases (case 1), *E. mundtii* was isolated from a chronic thigh abscess in a mixed culture together with *S. aureus* and, therefore, the disease-producing potential is possible but difficult to assess. In case 2, *E. mundtii* was also isolated from a normally sterile body site (operatively obtained sinus mucosa). However, this finding is interpreted as commensal colonization without evidence for invasive disease because of the negative histopathology.

Relying on commercially available identification systems, whose data bases do not include the more recent taxonomic changes, may lead to inaccurate identifications. In our described cases, the enterococcal strains were misidentified by both applied commercial systems because of the limitations described above. After description of the newer species, the strains were retrieved from storage and the correct differentiation was easily accomplished when attention was paid to certain key characteristics. The yellow pigment is more reliably observed if the colonies are picked up with a cotton swab. The species identification was confirmed by some conventional physiological tests mentioned in the suggested test scheme of Facklam and Collins (4).

The less frequently isolated enterococcal species, such as *E. mundtii*, seem not to differ in their overall antibiotic susceptibility from the clinically most important species, *E. faecalis* and *E. faecium*. It is therefore arguable whether accurate species identification of these isolates is justified in the routine laboratory. On the other hand, it is desirable in order to delineate their true incidence and their clinical significance.

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