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First Comprehensively Documented Case of *Paracoccus yeei* Infection in a Human

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***Paracoccus yeei* was isolated in pure culture from an aerobic blood culture and bulla fluid from a 67-year-old male. The biochemical identification scheme for this recently described species is outlined. Because of its reaction pattern it is not unlikely that *P. yeei* is underdiagnosed.**

CASE REPORT

A 67-year-old male patient was admitted to the emergency department of the hospital because of suspected pneumonia due to heart failure. On admission the patient was febrile (40.5°C), his leukocyte count was 13.8/nl, and his C-reactive protein level was 198 mg/liter. A sputum specimen was sent to the laboratory for culture, but no significant microorganisms grew. Shortly after admission, intravenous therapy with 1.5 g of cefazolin three times a day was initiated. Application of diuretics during the first 3 days of hospitalization led to improvement of the respiratory situation. Despite therapy with elastic bandages for congestion dermatitis (with ulcers due to pre-existing venous insufficiency), bullous lesions developed on his left leg. On day 4, a pair of blood culture bottles was prepared since the patient was still febrile. On the same day, a turbid fluid (moderate leukocytes in Gram stain) was aspirated from the largest bullae and sent to the laboratory for culture. After overnight incubation, gram-negative coccobacilli (which were arranged in small clusters) from both the aerobic blood culture bottle and the aspirated fluid grew in pure culture (see below). Because of the continuous improvement of the pulmonary situation and in accordance with susceptibility testing data, the antimicrobial therapy was changed to oral ofloxacin (400 mg twice a day) and maintained for 8 days. Diuretic therapy was continued for another 2 weeks. The patient underwent no further febrile episodes and was discharged with normalized levels of leukocytes and C-reactive protein.

Discussion. After subculturing from the aerobic blood culture bottle (BacTec Plus+Aerobic; BD, Sparks, Md.), whitish-greyish, convex colonies 0.5 to 1 mm in diameter grew on Columbia sheep blood agar and chocolate agar plates but not on MacConkey or colistin-nalidixic blood agar plates (all agar media were purchased from BD, Heidelberg, Germany). The features of the isolate from the aspirated fluid were absolutely identical to those of the blood culture isolate. The blood culture isolate was strongly oxidase positive, suggestive of *Neisseria* sp. or *Moraxella* sp. and excluding assignment to the genus *Acinetobacter*. The small rod forms revealed by Gram staining made assignment to the genus *Neisseria* (except *Neisseria weaveri* and *N. elongata*) or *Moraxella* unlikely. The isolate was catalase positive (weak reaction), nonmotile (hanging-drop method), indole negative, and an oxidizer (detected by using OF-glucose). Biotyping with the commercial API 20NE kit and database release 6.0 (bioMérieux, Marcy l'Etoile, France) revealed the numerical code 1101044 (positive reactions, nitrate reduction, arginine dihydrolase, and assimilation of arabinose and malate; negative reactions, indole, urease, esculin hydrolysis, gelatinase, β-galactosidase, assimilation of glucose, mannose, mannitol, *N*-acetylglucosamine, maltose, gluconate, caprate, adipate, citrate, and phenylacetate), which corresponded to an identification ("low selectivity" score) as either *Comamonas testosteroni*, *Pseudomonas alcaligenes*, *Pasteurella* sp., or *Methylobacterium mesophilicum*. Application of the commercial API NH gallery (bioMérieux) resulted in the numerical code 3600 (positive reactions, acid production from glucose and fructose, ornithine decarboxylase, and urease; negative reactions, acid production from maltose and sucrose, lipase, alkaline phosphatase, β-galactosidase, proline arylamidase, γ-glutamyltransferase, and tryptophan deaminase), corresponding to an identification ("doubtful" score, database release 2.0) as either *Haemophilus influenzae*, "*H. somnus*," or *H. parainfluenzae*.

Because of the initial lack of growth on MacConkey agar we considered the unknown bacterium to be a member of the group of fastidious gram-negative rods. Assignment to the genus *Pasteurella*, *Cardiobacterium*, or *Haemophilus* was unlikely because the isolate was not a fermenter. Macroscopic morphology made assignment to the genus *Actinobacillus*, *Eikenella*, *Kingella*, or *Streptobacillus* unlikely. *Capnocytophaga* spp. were ruled out because of the appearance of our isolate on Gram staining. Optimal growth was detected at 35°C rather than at 20 to 25°C, excluding assignment of the unknown isolate to *Psychrobacter immobilis* (4). After 72 h of incubation the isolate grew poorly on MacConkey agar plates; on Columbia sheep blood agar the whitish-greyish colonies developed into very mucoid beige colonies. The O-shaped Gram stain morphology (i.e., vacuolated or peripherally stained cells) described for the previous CDC group EO-2 bacteria (6) was not

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observed. In order to reveal the identity of the unknown isolate we sequenced its almost entire 16S rRNA gene (rDNA).

Three single colonies were suspended together in 0.9% DNA-free sodium chloride solution and centrifuged for 5 min at 16,000 × *g*. The supernatant was discarded by aspiration, and the pelleted bacteria were resuspended in 50 μl of 10 mM Tris/HCl (pH 9.0) and boiled for 15 min in capped Eppendorf tubes. After a brief centrifugation step, 5 μl of unpurified bacterial DNA solution was added to give a final PCR mixture of 50 μl. The PCR mixture contained 1× QIAGEN PCR buffer (with a final concentration of 1.5 mM $MgCl_2$), 2.5 U of *Taq* polymerase (QIAGEN, Hilden, Germany), a 200 μM concentration of each deoxynucleoside triphosphate, and a 0.4 μM concentration each of primers TPU-1 (5′-AGAGTTTGATC MTGGCTCAG-3′; nucleotides 8 through 27; sense) and 1492RPL (5′-GGTTACCTTGTTACGACTT-3′; nucleotides 1509 through 1491; antisense). The numbering of primers TPU-1 and 1492RPL refers to the nucleotide sequence of the *Escherichia coli* 16S rRNA deposited under EMBL-GenBank accession number J01859. All of the oligonucleotides used in the present study were synthesized at MWG Biotech, Ebersberg, Germany. The PCR assay included an initial step of denaturation at 95°C for 2 min; 35 cycles of 95°C for 45 s, 50°C for 30 s, and 72°C for 2 min; and a final step of elongation at 72°C for 10 min and was performed on a thermal cycler (Mastercycler gradient; Eppendorf, Hamburg, Germany). After gel electrophoresis and ethidium bromide staining, the amplification product was visualized under UV light. A single PCR product with a size of approximately 1.5 kb was obtained and purified with the MSB Spin PCRapace Kit (Invitek, Berlin, Germany) in accordance with the supplier's instructions. The purified PCR product was sequenced in both directions by primer walking with the following oligonucleotides as primers: sense, TPU-1 (5′-AGAGTTTGATCMTGGCTCAG-3′), TPU-2 (5′-CCARACTCCTACGGGAGGCA-3′), TPU-3 (5′-CAGCMGCCGCGGTAATWC-3′), TPU-4 (5′-GGATTAGA TACCCTGGTAGTCC-3′), TPU-5 (5′-AAACTYAAAKGAA TTGACGG-3′), and TPU-6 (5′-GGGCKACACACGTGCTA CAAT-3′); antisense, RTU-2 (5′-TGCCTCCCGTAGGAGT YTGG-3′), RTU-3 (5′-GWATTACCGCGGCKGCTG-3′), RTU-4 (5′-TACCAGGGTATCTAATCCTGTT-3′), RTU-5 (5′-CCGTCAATTCMTTTRAGTTT-3′), RTU-6 (5′-ATTGT AGCACGTGTGTMGCCC-3′), RTU-7 (5′-ACAAGRCCCG GGAACGTATT-3′), and 1492RPL (5′-GGTTACCTTGTTA CGACTT-3′). Sequencing was performed on an ABI PRISM 310 sequencer (Applied Biosystems, Darmstadt, Germany) with the BigDye Terminator v1.1 cycle sequencing kit (Applied Biosystems). Sequencing PCR products were purified with the DyeEx 2.0 Spin Kit (QIAGEN) as recommended by the supplier. The full-length sequence was determined by aligning overlapping DNA sequences with the Lasergene 5 Package (DNASTAR Inc., Madison, Wis.). The resulting 1,407-bp sequence has been deposited in the GenBank-EMBL database. The sequence was aligned and compared with all of the eubacterial 16S rDNA sequences available in the GenBank-EMBL database by using the nucleotide-nucleotide BLAST software from the National Center for Biotechnology Information (Bethesda, Md.). The best match was observed for the *Paracoccus yeei* strain G1212 (the type strain of this species) and G6155 16S rDNA sequences (GenBank-EMBL accession numbers AY014173 and AY014170, respectively). In the 1,407 bp compared, only two mismatches (position 1362, A instead of G; position 1368, T instead of A) and two gaps (positions 1361 and 1367) were detected. The 16S rDNA sequence homology of greater than 99.7% unambiguously demonstrated that the isolate described is a true member of *P. yeei*. The closest phylogenetic neighbors (except *P. yeei* strains) were found to be *Paracoccus* sp. strain T231 (97.4% 16S rDNA homology; accession number AF376134) and *P. denitrificans* DSM 413 (97.2% 16S rDNA homology; accession number Y16928). By applying a commercial random amplification of polymorphic DNA assay kit (REP_{PRO} DNA Fingerprinting; Bacterial BarCodes Inc., Houston, Tex.), we demonstrated that the two isolates from the blood culture and the aspirated fluid were of clonal origin.

For detection of the antimicrobial susceptibility pattern, disk diffusion testing on plain Mueller-Hinton agar plates was performed in accordance with NCCLS methodology (7). The *P. yeei* isolate described was susceptible to ampicillin (10-μg disk), ampicillin-sulbactam (10- and 10-μg disk, respectively), amoxicillin-clavulanic acid (20- and 10-μg disk, respectively), piperacillin-tazobactam (100- and 10-μg disk, respectively), cefazolin (30-μg disk), cefuroxime sodium (30-μg disk), cefepime (30-μg disk), ceftazidime (30-μg disk), cefaclor (30-μg disk), imipenem (10-μg disk), meropenem (10-μg disk), gentamicin (10-μg disk), amikacin (30-μg disk), tobramycin (10-μg disk), doxycycline (30-μg disk), ciprofloxacin (5-μg disk), and ofloxacin (5-μg disk).

Recently, Daneshvar et al. have described the former CDC group EO-2 bacteria as *P. yeeii* (2); the specific epithet was later changed to *yeei* in accordance with the international code for bacterial nomenclature (3). This is the first species belonging to the genus *Paracoccus* that has been described as being isolated from humans. Paracocci have their natural habitat in soil and brines (5) and are known for their physiological versatility (1). The genus *Paracoccus* comprises 17 species of aerobic, gram-negative coccobacilli that are catalase and oxidase positive, reduce nitrate, and are nonmotile (5; http://www.bacterio.cict.fr/p/paracoccus).

The strain described in the present report is the first from a country other than the United States or Canada. Our strain represents only the second blood culture isolate described in the literature and the first comprehensively documented case of a human infection because the clinical data given in the original description from two reference centers were limited (2). In addition, the present report outlines *P. yeei* antimicrobial susceptibility data and numerical codes for commercial identification systems for the first time. The discrepancy in urease activity testing in both the API NH and API NE systems might be explained by the stronger buffering capacity in the API NE well. Workers from the Centers for Disease Control and Prevention described only one of eight *P. yeei* isolates as ornithine decarboxylase or arginine dihydrolase positive (2), as was our isolate in the test systems used.

The source of the *P. yeei* strain remained unclear. Unfortunately, no blood cultures had been taken on admission so that it is unknown whether the patient was bacteremic because of *P. yeei* at that time. However, in vitro testing demonstrated that the *P. yeei* strain was susceptible to cefazolin, thereby making an initial *P. yeei* bacteremia unlikely. *P. yeei* may have gained

access to the patient's body through the pre-existing leg ulcers either before or during hospitalization.

The pathogenic potential of paracocci seems to be limited if the frequency of paracocci in soil and the number of published case reports on this genus are considered. Despite its low pathogenic potential, we believe that *P. yeei* is probably underdiagnosed. This is, in part, due to its macroscopic appearance with colonies initially resembling those of a coagulase-negative staphylococcus (CNS). If a Gram stain or an oxidase reaction test is not performed by chance, then misidentification of *P. yeei* as a CNS may occur, in particular because of the delayed growth of *P. yeei* on MacConkey agar plates (2, 8, 9). In addition, a suspected CNS with susceptibility to nearly all of the antimicrobial agents tested may not trigger any further biochemical investigations for species identification. In the present case application of molecular genetic methods to our blood isolate rapidly led to its unambiguous identification. As the methods for molecular identification of bacteria are being increasingly used, it is expected that more reports on *P. yeei* infections may appear in the literature in the near future.

Nucleotide sequence accession number. The 16S rDNA sequence of the isolate described has been deposited in the GenBank database under accession no. AY528674.

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