

is critical. If clinicians suspect coccidioidomycosis, laboratory personnel should be alerted in order to reduce the risk of accidental exposure, since cultures of *Coccidioides immitis* must be handled with appropriate containment.

With the increasing number of Europeans traveling to endemic areas, it is important for clinicians to consider coccidioidomycosis, particularly in HIV-infected patients with a CD4+ lymphocyte count below 250/mm³.

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Enterococcus cecorum Septicemia in a Malnourished Adult Patient

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Enterococcus cecorum, a species typically isolated from chicken, pigs, calves, horses, ducks, cats, dogs, and canaries, was isolated from the blood of a patient with severe septicemia. The isolate was identified by conventional biochemical tests. Identity as *Enterococcus cecorum* was confirmed by SDS-PAGE analysis of whole cell protein. This is the first report of the isolation of *Enterococcus cecorum* in a clinical setting.

Enterococcus cecorum was first isolated from chicken intestines and described as *Streptococcus cecorum* in 1983 (1). It was reclassified as *Enterococcus cecorum* (2) based on the results of reverse transcriptase sequencing of its 16S ribosomal RNA. The species was further isolated from the intestines of pigs, calves, horses, ducks, cats, and dogs and from the crop of canaries (3–7). To our knowledge, *Enterococcus cecorum* has never been described in a clinical setting. Recently, this bacterium was isolated from two sets of blood cultures obtained from a 44-year-old woman who presented with symptoms and signs of septic shock.

Case Report. A 44-year-old woman was admitted to our hospital on 4 August 1995 for treatment of dehydration. At presentation she also had mucositis, cheilitis, glossitis, alopecia, diarrhea, and osteoporosis secondary to vitamin deficiency. In the past the patient had undergone numerous surgi-

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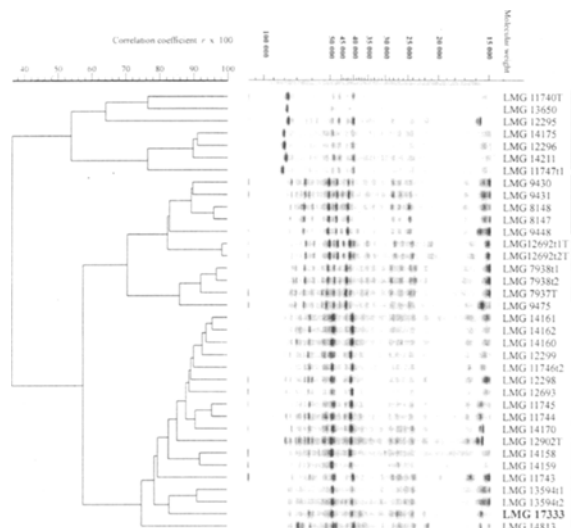


Figure 1: SDS-PAGE profiles of whole-cell extracts of different enterococci, including isolate 7504 (LMG 17333). Far left section: The mean correlation coefficient, expressed as a percentage ($r \times 100$) and represented as a dendrogram, calculated by the unweighted average pair grouping method for a number of *Enterococcus* spp. Points 20 to 325 of the 400-point traces, indicated by a gray bar on the molecular weight axis, were used for the calculation of similarities between the individual pairs of traces. The middle part of the figure represents a computer-processed printout of positions 0 (top of the gel, left-hand side of the pattern) to 360 (bottom of the gel, right-hand side of the pattern) of the digitized and normalised protein patterns of all strains compared. Right section: LMG numbers (Culture Collection of the Laboratory of Microbiology Gent, University of Gent, Gent, Belgium) and cluster delineation per species. T indicates type strain; t1 & t2, two different colony types.

cal procedures for morbid obesity (digestive bypass, gastroplasty, surgical treatment of complications of previous operations). During the summer of 1995, she spent most of her time in the company of her three cats and one dog. Three weeks before admission, she presented with a worsening of her general status and multiple skin lesions located predominantly on her hands, feet, and sacrum, assumed to be due to malnutrition secondary to intestinal malabsorption and a loss of appetite.

Seven days after admission, the patient developed tachypnea (25/min), tachycardia (120/min), hypotension (blood pressure 85/55 mmHg), and mild fever (37.4°C). Severe sepsis with hypotension was suspected and subsequently confirmed by paraclinical parameters: lactic acidosis (lactate 2.7 mmol/l), hypotension with normal central venous pressure (8 mmHg), low systemic resistance (583 dyn /sec \times cm⁻⁵), and high cardiac output (8.09 l/min). Intravenous norepinephrine (4–8 μ g/min)

was begun in order to stabilize the systemic arterial pressure.

Two blood cultures were taken on day 7 after admission through a central jugular i.v. line in place for less than 2 h. A gram-positive coccus grew after two days of incubation in four biphasic blood culture bottles (Septi-Check; Becton-Dickinson, USA). The organism was susceptible to ampicillin, ciprofloxacin, imipenem, and vancomycin, as determined by the Kirby-Bauer disk diffusion method.

Imipenem was administered intravenously for nine days, followed by ciprofloxacin for five more days. The patient's fever decreased, and thereafter, the hemodynamic variables improved rapidly. Blood cultures taken on day 9 after admission from three different intravascular catheters (arterial, side-arm, and Swan-Ganz) remained sterile. The catheters were not cultured when removed. The septicemia was not explained by any other infectious or noninfectious origin.

The turbidity of all four blood culture broths was increased, and small bacterial colonies were seen on the chocolate agar side of the Septi-Check slide. Microscopic examination of gram-stained blood culture smears revealed short chains composed of about four gram-positive cocci. Catalase, pyrrolidonyl peptidase, and group D antigen reactions, performed on the slide's colonies, were all negative. Different solid agar media were inoculated. Growth of small colonies was obtained from all four bottles on chocolate agar, Columbia blood agar, and sheep blood agar after one day of incubation in a CO₂ atmosphere and on supplemented blood agar under anaerobic conditions. The isolate, assigned number 7504, formed small, smooth, regular, gray, and bulgy colonies (1–2 mm in diameter) that were slightly alpha-hemolytic on sheep blood agar. No growth was obtained after overnight incubation in bile-esculin medium and in 6.5% NaCl broth. Catalase, pyrrolidonyl peptidase, and group D antigen reactions were still

Table 1: Characteristics differentiating isolate 7504 from main groups of enterococci (adapted from Reference 9).

	Reaction (% positive)			
	Group 1	Group 2	Group 3	Isolate 7504
Mannitol	+ (100)	+ (99)	- (7)	+
Sorbitol	+ (97)	v (63)	- (0)	-
Sorbose	+ (97)	- (0)	- (0)	-
Arginine	- (0)	+ (94)	+ (100)	-

+, positive reaction; -, negative reaction; v, variable.

negative even when colonies of overnight cultures were tested.

The Rapid ID 32 Strep (bioMérieux, France) was used to identify the isolate. After 4 h of incubation under normal aerobic conditions, positive reactions were found for Voges Proskauer, alkaline phosphatase, beta-glucuronidase, alpha-galactosidase, beta-galactosidase, trehalose, raffinose, maltose, ribose, saccharose, cyclodextrine, methyl B-D-glucopyranoside, melibiose, melezitose, and glycyL tryptophane arylamidase but negative reactions for pyrrolidonylarylamidase, hippurate, L-arabinose, mannitol, sorbitol, leucine arylamidase, arginine dihydrolase, D-arabitol, alanyl-phenylalanine arylamidase, N-acetyl-beta glucosaminidase, glycogen, puliulane, beta-mannosidase, and urease. Lactose and tagatose reactions were indeterminate.

With the API ZYM (bioMérieux), which confirmed most of the results obtained with Rapid ID 32 Strep, the isolate reacted positively for leucine arylamidase, alpha-glucosidase, acid phosphatase, esterase, esterase-lipase, and alpha-chymotrypsin but negatively for beta-glucuronidase, alpha-mannosidase, lipase, valine, arylamidase, cystine, trypsin, naphthol-AS-BI-phosphohydrolase, and alpha-fucosidase.

Growth on agar plates was better when the plates were incubated in 10% CO₂, but CO₂ was not absolutely required, as growth was also observed in an aerobic atmosphere without CO₂ on sheep blood agar. The isolate also was facultatively anaerobic, unable to grow in NaCl 6.5% even when incubated for ten days with and without CO₂, unable to grow on bile-esculin medium without CO₂, but able to grow poorly on bile-esculin medium when incubated in 10% CO₂, and able to grow on esculin agar with and without CO₂.

These reaction and growth patterns suggested the isolate was *Enterococcus cecorum*. Further testing performed in the laboratory of the University of Gent produced the following results. The isolate was nonmotile (motility test agar), amylase negative, and did not grow on Slanetz and Bartley enterococcal selective medium. In the API 20 Strep test (bioMérieux), which confirmed most of the results obtained with the Rapid ID 32 Strep, the isolate presented additional positive reactions for esculin/beta-glucosidase, lactose, and starch. Leucine arylamidase was weakly positive, beta-glucuronidase indeterminate, and mannitol negative. With Rosco diagnostic tablets (Rosco, Denmark), the isolate was alkaline phosphatase

Table 2: Comparison of characteristics of *Enterococcus avium*, *Streptococcus bovis*, *Enterococcus columbae*, *Enterococcus cecorum*, and isolate 7504 (adapted from Reference 2).

	Beta-glucuronidase	Alkaline phosphatase	Production of acid	
			L-arabinose	Methyl-D-glucoside
<i>E. avium</i>	-	-	+	+
<i>S. bovis</i>	-	-	-	-
<i>E. columbae</i>	-	+	+	+
<i>E. cecorum</i>	+	+	-	-
Isolate 7504	+	+	-	-

+, positive reaction; -, negative reaction.

and beta-glucuronidase positive. A weak reaction was produced in the beta-galactosidase test.

In the API 50 carbohydrate tests (bioMérieux), the isolate presented positive reactions for ribose, trehalose, inulin, raffinose, lactose, maltose, melibiose, melezitose, D-glucose, galactose, D-fructose, D-mannose, N-acetyl glucosamine, amygdalin, arbutin, esculin, salicin, cellobiose, sucrose, and beta-gentiobiose and negative reactions for D-arabinose, sorbitol, glycogen, L-arabinose, D-arabitol, D-tagatose, glycerol, erythritol, D- and L-xylose, 2- and 5-ketogluconate, adonitol, beta-methylxyloside, L-sorbose, rhamnose, dulcitol, inositol, alpha-methyl-D-mannoside, alpha-methyl-D-glycoside, starch, xylitol, D-turanose, D-lyxose, D- and L-fucose, and gluconate. Acid was produced from D-mannitol with a delay.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis of whole cell protein confirmed the identity of isolate 7504 (LMG 17333) as *Enterococcus cecorum*, with a pattern closely related to that of *Enterococcus cecorum* strains of animal origin (Figure 1). Its SDS-PAGE profile was significantly different from that of other enterococci, including that of its closest biochemical relative, *Enterococcus columbae*.

The *Enterococcus cecorum* isolate (7504) gave a negative result with a commercially available nucleic acid probe for enterococci (AccuProbe; Gen-Probe, USA), while an *Enterococcus faecalis* strain (ATCC 19433), an *Enterococcus faecium* strain (ATCC 14025), and an *Enterococcus avium* strain (ATCC 19434) used as positive controls gave positive results, and a *Streptococcus bovis* strain (ATCC 33317) used as negative control gave a negative result.

Discussion. Comparative analysis of 16S ribosomal RNA sequences of 11 *Enterococcus* species determined by reverse transcription revealed the presence of three species groups within the genus. The species *Enterococcus faecium*, *Enterococcus durans*, *Enterococcus hirae*, and *Enterococcus mundtii* formed a distinct group, as did *Enterococcus avium*, *Enterococcus pseudoavium*, and *Enterococcus raffinosus* and the pair of species *Enterococcus casseliflavus* and *Enterococcus gallinarum*. Of the remaining species, *Enterococcus faecalis*, *Enterococcus cecorum*, *Enterococcus columbae*, and *Enterococcus saccharolyticus* formed distinct lines of descent within the genus (8). Our isolate proved to be an uncommon enterococcus, as it was unable to grow in NaCl broth and grew poorly in bile-esculin solid media. Furthermore, the negative pyrrolidonylarylamidase reaction is atypical for enterococci. When we tried to classify our isolate into one of the three enterococcus groups defined by phenotypic key tests (9), we found that its negative reactions for sorbitol, sorbose, and arginine and its positive reaction for mannitol made classification difficult (Table 1).

Even if the growth of this isolate is similar to that of *Streptococcus bovis*, *Enterococcus cecorum* differs mainly in the absence of group D antigen, positive alkaline phosphatase, and positive beta-glucuronidase and in the production of acid in API 50 carbohydrate tests from melezitose and ribose. Our isolate resembles *Enterococcus columbae* and many *Enterococcus avium* strains in its lack of group D antigen and in being more susceptible to NaCl than other enterococci. It differs from the classical biochemical profile of *Enterococcus avium* in its negative reactions for pyrrolidonylarylamidase, arginine, sorbitol, rhamnose, L-arabinose, and D-arabitol. *Enterococcus cecorum* can be differentiated (7) and has been differentiated from *Enterococcus columbae* strains by its negative L-arabinose and positive beta-glucuronidase reactions. Thus, characterization of the isolated organism with conventional biochemical tests strongly suggested it to be *Enterococcus cecorum* (Table 2). Analysis of whole cell protein extracts (SDS-PAGE) confirmed this identification. Indeed, the SDS-PAGE profile of isolate 7504 (LMG 17333) is clearly different from that of other known species of enterococci, including *Enterococcus columbae*. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of whole cell protein extracts is used increasingly for the identification of gram-positive lactic acid bacteria, and its accuracy was recently established (10–12).

The commercially available nucleic acid probe for enterococci (AccuProbe; Gen-Probe) was designed to react with enterococci of human origin. Thus, as demonstrated with this isolate, *Enterococcus cecorum* could be missed if the probe method were used to identify isolates from human infection.

We hypothesize that our patient acquired this strain from one of her domestic animals through cutaneous lesions secondary to malnutrition. After an initial period of asymptomatic colonization, this microorganism reached the bloodstream, possibly via placement of an intravenous catheter. There, it caused severe septicemia with hypotension, which could not be explained by any other infectious or noninfectious origin.

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Mixed *Candida glabrata* and *Candida albicans* Disseminated Candidiasis in a Heroin Addict

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The case of a white-heroin addict who developed disseminated candidiasis following coinfection by *Candida glabrata* and *Candida albicans* is reported. Genomic random amplified polymorphic DNA typing suggested that the *Candida glabrata* blood isolates originated in the oral cavity of the patient. This case strengthens the evidence that *Candida* species other than *Candida albicans* can be involved in the pathogenesis of disseminated candidiasis in heroin addicts.

Disseminated candidiasis is a syndrome comprising cutaneous, ocular, and osteoarticular lesions, first described in 1981 in addicts using brown sugar heroin diluted in lemon juice (1). The source of infection, however, remained unclear. Some authors postulated that the disseminated infection originated directly from yeasts colonizing the intestinal tract of the patient (2), while others suggested that the vehicle of infection was the lemon juice, which might have been contaminated by the yeasts colonizing the addict before the heroin was injected (3, 4). Whatever the source of infection, only cases due to *Candida albicans* have been reported thus far (1–3, 5, 6).

We report the case of a white heroin addict infected with the human immunodeficiency virus (HIV) who developed disseminated candidiasis. Isolates of *Candida glabrata* were obtained from both blood and the oral cavity and isolates of *Candida albicans* from both cutaneous lesions and the oral cavity.

Case Report. A 30-year-old male drug user, known to be seropositive for both HIV and hepatitis C virus, was admitted to our hospital on 18 March 1996. Two days before hospitalization, he had experienced a transient episode of fever 10 min after a heroin injection. The injected drug was so-called “white stuff”. Usually, the patient dissolved the heroin in tap water in a spoon and then sucked the fluid into a syringe through a cigarette filter before injection. The used filters were stored in a small box for a few days before being used. For the injection related to the current episode, the drug was recovered from the used filters by rehydrating them with water. No lemon juice or any other acidic solvent was used. At admission, the patient complained of headache, shivering, myalgia and profuse sweating. Physical examination revealed no neurological deficit or meningeal signs. The results of cerebrospinal fluid analysis and cerebral computed tomography scan were normal. A chest radiograph was also normal. Leukocyte count was 6700/mm³, and CD4+ cell count was 273/mm³. Liver enzyme levels were elevated: glutamyl transferase, ASAT, and ALAT levels were five, two, and three times higher than normal, respectively. Blood cultures drawn on days 1 and 2 were positive for *Candida glabrata*.

Two days after admission, pustules and folliculitis were observed in the beard area and scalp of the patient. Cultures of these cutaneous lesions yielded *Candida albicans*. Oral cavity swabs performed on day 3 were positive for both *Candida albicans* and *Candida glabrata*, although no oral

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