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Leuconostoc pseudomesenteroides as a Cause of Nosocomial Urinary Tract Infections

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The phenotypic and genotypic characterization of five clinical isolates of *Leuconostoc pseudomesenteroides* associated with nosocomially acquired urinary tract infections is described. All the strains were susceptible to chloramphenicol, clindamycin, erythromycin, gentamicin, and tetracycline; all were resistant to nalidixic acid, norfloxacin, and vancomycin; and all were intermediately affected by ampicillin and penicillin. Analysis of chromosomal DNA by pulsed-field gel electrophoresis after treatment with *Sma*I indicated a clonal relationship of the isolates. The results provide evidence for the possibility of nosocomial transmission of this unusual opportunistic, vancomycin-resistant pathogen.

The genus *Leuconostoc* is composed by catalase-negative gram-positive microorganisms with irregular coccoid morphology. These organisms may be misidentified as *Lactobacillus*, *Streptococcus* (particularly the viridans group), *Pediococcus*, or even *Enterococcus*, as all share several biochemical properties (3, 20). Unlike most other gram-positive bacteria, these microorganisms have an important physiological marker related to their intrinsic resistance to vancomycin (6, 18). Before 1985, *Leuconostoc* species were usually considered nonpathogenic and, therefore, of little or no importance in clinical microbiology (3, 19). Since then, increasing numbers of infections due to *Leuconostoc* have been reported (1, 2, 8–12, 23). Despite remaining uncommon, these pathogens are gaining importance as opportunistic agents of human infections associated with high mortality rates, mainly bacteremia (14, 15, 18). Infections due to *Leuconostoc* occur more frequently in patients being treated for underlying diseases with vancomycin therapy (7, 13), although *Leuconostoc* infections have also been documented in otherwise healthy patients (4). The present study describes the phenotypic and genotypic characterization of a cluster of five *Leuconostoc pseudomesenteroides* strains recovered from hospitalized patients with symptomatic urinary tract infections, providing evidence for the possible nosocomial transmission of this opportunistic vancomycin-resistant bacterium.

Five clinical isolates of catalase-negative, vancomycin-resistant, gram-positive cocci recovered from urine specimens obtained from five inpatients admitted to a University Hospital in Rio de Janeiro, Brazil, were studied. The strains were isolated within a period of 1 week (in April 1997) from patients in two units (nephrology and urology) located on the same hospital floor. Clinical manifestations of the infections included dysuria and/or fever, and the microorganisms grew in pure cultures. All five patients had been admitted to the hospital due to other medical conditions, and only one of the patients had a urinary catheter at the time the culture-positive urine was collected. The most common risk factors associated with infection acquisition are described in Table 1.

Identification of the strains to the genus level was performed as described elsewhere (6) by using tests for detecting the following physiological characteristics: presence of catalase, pyrrolidonyl arylamidase and leucine aminopeptidase activities, hydrolysis of esculin in the presence of bile, growth in the presence of 6.5% NaCl, vancomycin susceptibility, and production of gas in lactobacilli De Mann, Rogosa, and Sharp (MRS; Difco Laboratories, Detroit, Mich.) broth. Additional physiological tests, including production of acids from arabinose, lactose, maltose, melibiose, salicin, sucrose, threalose, and xylose, were used for the characterization of the isolates to the species level. All five clinical isolates had similar physiological characteristics. They were negative for catalase, pyrrolidonyl arylamidase, and leucine aminopeptidase activities and did not grow in broth containing 6.5% NaCl. They all were resistant to vancomycin, were esculin-positive in bile, produced gas in MRS broth, and produced acid from arabinose, lactose, maltose, melibiose, salicin, sucrose, threalose, and xylose. On the basis of these results, the most likely identity of the isolates was *L. pseudomesenteroides*. The isolation of *L. pseudomesenteroides* from human clinical specimens is rare, and, to the best of our knowledge, there are no specific reports of its association with urinary tract infections. The majority of *Leuconostoc* strains associated with human infections have been identified as *Leuconostoc mesenteroides*, followed by *Leuconostoc lactis* and *Leuconostoc citreum* (5, 6). On the other hand, the discrimination between species of *Leuconostoc* is often problematic, and the description of the role of each individual species as infectious agent has possibly been hindered by the difficulty of precise identification. Differentiation of *L. pseudomesenteroides* and the most frequent species, *L. mesenteroides*, is mainly based on the results of growth in 6.5% NaCl, a test which is sometimes difficult to interpret and to reproduce (5, 6).

To confirm the identification of the isolates, analysis of whole-cell protein profiles using one-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis was performed as described by Merquior et al. (16). This method has been considered to be a reliable and reproducible tool for the differentiation and identification of several species of catalase-negative, gram-positive cocci, including *Leuconostoc* spp. (5,

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TABLE 1. Characteristics of patients with urinary tract infections caused by *L. pseudomesenteroides*

Patient no.	Age (yr), sex[a]	Underlying conditions	Previous therapy	Associated symptoms	Concn of organisms in urine (CFU/ml)	Clinical outcome
1	16, M	Hydrocephalus and prolonged hospital stay	Vancomycin and cephalosporin	Fever	5×10^5	Death
2	29, M	Asthma, alcoholism, and drug addiction	None	Dysuria and fever	5×10^5	Recovery
3	14, F	Renal transplantation and prolonged hospital stay	Vancomycin and trimethoprim-sulfametoxazol	Dysuria	3×10^5	Recovery
4	18, F	Endometriosis and prolonged hospital stay	Vancomycin and cephalosporin	Dysuria and fever	4×10^5	Recovery
5	37, F	Not determined	None	Dysuria and fever	4×10^5	Recovery

[a] M, male; F, female.

21, 22). Protein profiles were compared and clustered by the unweighted pair group method with averages by using the Molecular Analyst Fingerprint Plus software of the Image Analysis System (Bio-Rad Laboratories, Richmond, Calif.). The clinical isolates had virtually indistinguishable protein profiles (Fig. 1) and had higher similarity (average similarity, 89%) with the profile of the *L. pseudomesenteroides* type strain (SS 1292, ATCC 12291) than with that of the *L. mesenteroides* type strain (SS 1238, ATCC 8293). These findings confirmed the identification based on conventional physiological tests and indicate that analysis of whole-cell protein profiles can be recommended as an additional tool for the precise identification of *L. pseudomesenteroides*.

MICs were determined by the microdilution method according to the recommendations of the National Committee for Clinical Laboratory Standards for *Streptococcus* spp. other than *Streptococcus pneumoniae* (17), since no criteria are specified for *Leuconostoc* strains. Results indicated that the clinical isolates were susceptible to chloramphenicol (MIC = 8 μg/ml), clindamycin (0.015 μg/ml), erythromycin (0.1 μg/ml), gentamicin (0.12 μg/ml), and tetracycline (4 μg/ml) and were resistant to nalidixic acid (MIC = 128 μg/ml), norfloxacin (32 μg/ml), and vancomycin (512 μg/ml). Intermediate results were obtained for ampicillin (MIC = 2 μg/ml) and penicillin (1 μg/ml). No strain-to-strain variation in the MICs was observed.

The genotypic relationship of the strains was investigated by analysis of *Sma*I-digested chromosomal DNA by pulsed-field gel electrophoresis (PFGE) based on the procedure recommended by Teixeira et al. (21). The following parameters for electrophoresis were used: voltage gradient, 6 V/cm; running time, 22 h; temperature, 11°C; pulse time, ramping from 2 to 25 s; and included angle, 120°. The PFGE patterns of all the isolates were found to be identical, and they were distinct from the pattern obtained for the *L. pseudomesenteroides* type strain (Fig. 2). These data suggested that the isolates may have originated from a common source.

In conclusion, this report presents the phenotypic and genotypic characterization of a cluster of five *L. pseudomesenteroides* strains associated with nosocomially acquired urinary tract infections. Taken together, the isolation, within a short period of time, of such a rare opportunistic bacterial species, in pure cultures and in significant numbers, from the urine of symptomatic patients in two related hospital units, in association with the results of genotypic characterization of the isolates, provided evidence of the outbreak potential and the risk of possible nosocomial transmission of this vancomycin-resistant bacterial species. With the increased use of vancomycin, infections due to vancomycin-resistant microorganisms, such as *Leuconostoc*, may be more frequently encountered, especially in debilitated individuals. The observation of the outbreak described in this paper highlights the need for clinical laboratories to be aware of the potential clinical significance of these bacteria and to be prepared to promptly and accurately identify them. The incorporation of precise procedures

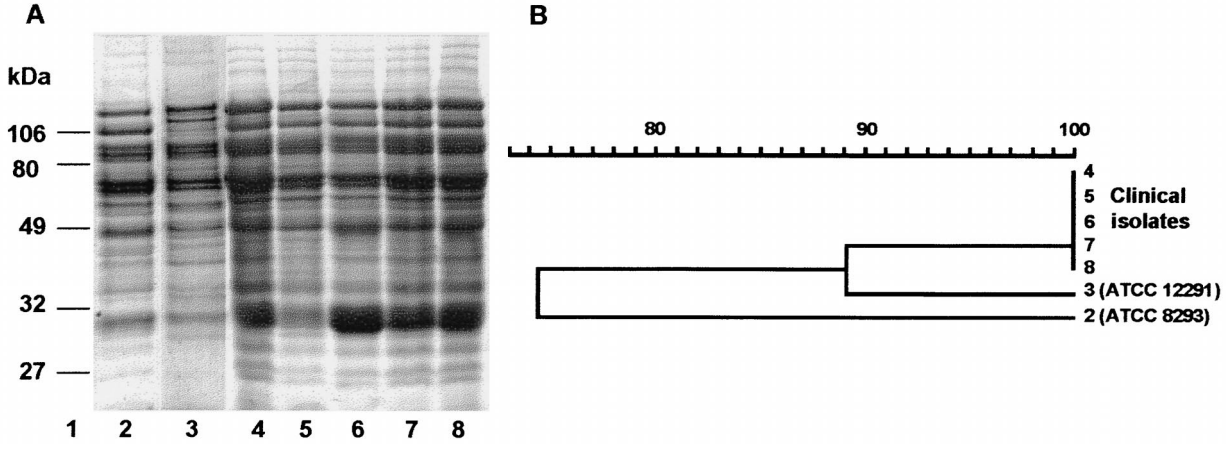

FIG. 1. (A) Sodium dodecyl sulfate-polyacrylamide gel electrophoresis profiles of whole-cell protein extracts of *Leuconostoc* strains. Lane 1, molecular mass markers (in kilodaltons); lane 2, *L. mesenteroides* ATCC 8293; lane 3, *L. pseudomesenteroides* ATCC 12291; lanes 4–8, clinical isolates of *L. pseudomesenteroides*. (B) Dendrogram resulting from computer-assisted analysis of the protein profiles shown in panel A. The scale represents the average percentage of similarity.

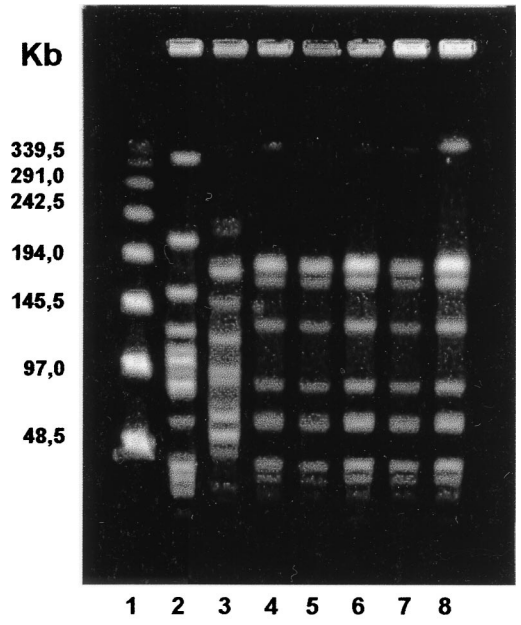

FIG. 2. PFGE patterns of chromosomal DNA of *Leuconostoc* strains after digestion with *Sma*I. Lane 1, molecular size markers (in kilobases); lane 2, *L. mesenteroides* ATCC 8293; lane 3, *L. pseudomesenteroides* ATCC 12291; lanes 4–8, clinical isolates of *L. pseudomesenteroides*.

into the catalase-negative, gram-positive coccus identification schemes and the application of molecular tools will allow proper detection and characterization of unusual pathogens such as *Leuconostoc* species, thereby improving our knowledge of the epidemiological aspects of human infections caused by these microorganisms and their routes of transmission.

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