Letter to the Editor

An Outbreak of Extended-Spectrum β-Lactamase–Producing *Klebsiella* Species in a Neonatal Intensive Care Unit in Brazil

To the Editor:

Strains of *Klebsiella* that produce extended-spectrum β-lactamases (ESBLs) constitute a persistent problem in many parts of the world, especially in intensive care units.[1] Moreover, these bacteria are increasing sources of resistance to third-generation cephalosporins.[1] Investigation of presumed outbreaks caused by *Klebsiella* species often requires strain typing data to identify outbreak-related strains, but phenotypic methods of identification are not always conclusive. Molecular typing techniques such as pulsed-field gel electrophoresis and polymerase chain reaction (PCR)–based techniques are the best methods available for strain delineation.[2]

During a recent *Klebsiella* outbreak in the neonatal intensive care unit (NICU) of the University Hospital in Londrina, southern Brazil, the isolated strains presented an uncommon biochemical phenotype. This prompted further investigation by means of fingerprinting generated by PCR with enterobacterial repetitive intergenic consensus (ERIC) sequences.[3]

In May 1998, atypical *Klebsiella* was isolated from the cerebrospinal fluid and blood of 1 patient in the NICU; 2 patients in that unit subsequently died of sepsis. Bacteria with similar phenotypic characteristics were concomitantly isolated from surveillance cultures of tracheal secretions from all 11 neonates in the unit. Two of 14 samples taken from the environment yielded positive results, as did 1 of 84 samples from the NICU staff. All patients were then transferred to another unit, the NICU was thoroughly disinfected, and staff training was reinforced. Once these control measures had been taken, the atypical *Klebsiella* apparently disappeared. However, it was isolated again several months later.

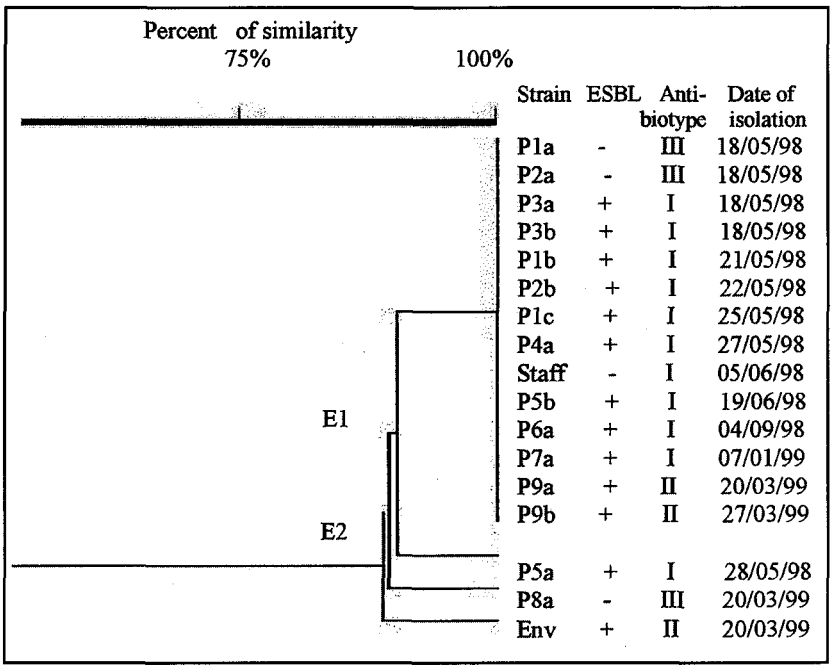

FIGURE. Genetic variation of extended-spectrum β-lactamase–producing *Klebsiella* (ESBL) species among isolates from a neonatal intensive care unit in Brazil. Dendrogram of isolates based on simple matching similarity coefficients, calculated from enterobacterial repetitive intergenic consensus–polymerase chain reaction analysis data.

We analyzed 17 strains from that outbreak by means of ERIC–PCR; 15 isolates were from the tracheal secretions of 9 neonates, 1 was from the nasopharynx of an NICU staff member, and 1 was from the NICU environment. Strains were identified using standard techniques, followed by a Microscan gram-negative panel performed according to the manufacturer's instructions (Microscan Dade, West Sacramento, CA). Susceptibility to antimicrobial agents was determined by the agar disk-diffusion method and by Microscan. The presence of ESBLs was determined by the double-disk synergy test.[1]

K. pneumoniae is usually identified by standard laboratory procedures, such as lack of motility, carbohydrate fermentation patterns, positive results on Voges-Proskauer test, urease and lysine decarboxylase production, and negative results on ornithine decarboxylase and indole tests. However, the isolates studied showed positive results on the ornithine decarboxylase test and negative results on the indole test, which impeded differentiation between the species *pneumoniae* and *ornithinolytica*. Thus, the species of bacteria responsible for the outbreak could not be established. All isolates were uniformly susceptible to imipenem and ciprofloxacin and were distributed into three antibiotypes according to their resistance pattern. The strains of antibiotype I were the most resistant, showing resistance to cephalothin, sulbactam-ampicillin, gentamicin, chloramphenicol, ceftazidime, aztreonam, cefotaxime, piperacillin-tazobactam, and cefepime. The strains of antibiotype II presented susceptibility to ceftazidime, aztreonam, and cefotaxime and were resistant to amikacin. The strains of antibiotype III were resistant to one antibiotic or susceptible to all antibiotics. Except for the strain from the staff (antibiotype I) and the strains that belonged to antibiotype III, all other strains showed production of ESBLs (Figure).

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ERIC–PCR of isolates was obtained with the oligonucleotides ERIC 1R (5'-ATGTAAGCTCCTGGGGATTC AC-3') and ERIC2 (5'-AAGTAAGT GACTGGGGTGAGCG-3'),[3] using 30 cycles at 90°C for 30 seconds, 45°C for 1 minute, and 65°C for 8 minutes, and 1 cycle at 68°C for 16 minutes. The ERIC–PCR patterns of each isolate were consistent on 3 different days. *Klebsiella* DNA generated patterns of 11 to 13 distinct amplification bands ranging from 0.08 to 1.6 kb. A dendrogram was constructed to show the degree of relatedness among the strains, by the unweighted pair group method with arithmetic (UPGMA) analysis and coefficient simple matching, based on ERIC–PCR fingerprints (Figure). Fourteen isolates, 13 obtained from patients and 1 obtained from a staff member, were allocated into cluster E1 with 100% similarity. Cluster E2 contained 3 isolates, 2 from patients and 1 from the environment, and presented more than 90% similarity to cluster E1. All strains isolated in 1998 were grouped in the same cluster (E1), with the exception of the P5a isolate (E2), which, nevertheless, presented more than 95% similarity to E1. Some isolates obtained in 1999 belonged to a cluster isolated in 1998, but unlike the 1998 isolates, the 1999 isolates were resistant to amikacin. The first 2 isolates obtained from 2 patients (P1a and P2a) were not producing ESBLs, but isolates obtained from those same patients 3 and 4 days later, respectively (P1b and P2b), were positive for ESBL, which suggests that exposure to cephalosporins induced expression of ESBLs.

ERIC–PCR is a fast method that clearly reveals the clonal relationships between strains, and has been used for molecular epidemiology of some medically important bacteria.[4] It was used in a study of an outbreak of cefoxitin-resistant *K. pneumoniae*, in which it was compared with pulsed-field gel electrophoresis and provided evidence of the clonal origin of the isolates.[5] In this study, the application of ERIC–PCR allowed us to characterize the genetic relationship among isolates from an outbreak of ESBL-producing *Klebsiella*, which in turn made possible a better evaluation of the effectiveness of the control measures adopted. There have been no new cases of infection by bacteria related to those strains of atypical *Klebsiella*, indicating that the control measures adopted were adequate.

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