Investigation of an Apparent Cluster of Klebsiella pneumoniae Bacteremias Using Random Amplified Polymorphic DNA Analysis

Author(s): James C. Hurley, Edward G. Russell, Glenys Harrington and W. John Spicer

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the preservative in albuterol, decreased in open bottles, probably because of adsorption to the plastic and rubber parts of the dropper and cap.[12]

A cost analysis revealed that the use of single-dose vials of albuterol would cost $33,800 per year more than the use of multidose vials. Antibiotic charges and infectious diseases consultant services alone added $52,400 to the cost of care for the patients involved in this outbreak. Because extrinsic contamination of solutions and resultant colonization and infection of patients has been recognized for many years, consideration should be given to the use of single-dose vials or patient-dedicated vials for nebulized solutions.

95-CC-181. Address reprint requests to Annette C. Reboli, MD, Cooper Hospital/University Medical Center, Division of Infectious Diseases, 401 Haddon Ave, Camden, NJ 08103.

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Investigation of an Apparent Cluster of *Klebsiella pneumoniae* Bacteremias Using Random Amplified Polymorphic DNA Analysis

James C. Hurley, MB, BS, PhD; Edward G. Russell, MSc; Glenys Harrington, RN, RM; W. John Spicer, MB, BS

ABSTRACT

A cluster of bacteremia episodes with *Klebsiella pneumoniae* was noted in patients in a hematology-oncology ward during a 3-week period. Random amplified polymorphic DNA (RAPD) analysis, a novel technique for generating chromosomal fingerprints from bacterial isolates, was used as an aid to the epidemiological investigation of this cluster. For each of the two patients from whom multiple isolates had been obtained, identical RAPD patterns were observed in the serial isolates, even for a patient where the isolates had different biotypes. Isolates from different patients gave distinct patterns. Random amplified polymorphic DNA was found to be a useful typing technique for this cluster of *K pneumonia* bacteremias (*Infect Control Hosp Epidemiol* 1996;17:743-745).

INTRODUCTION

We report here the epidemiological investigation of *Klebsiella pneumoniae* isolates obtained during a 3-week period from five patients in the hematology-oncology ward and a patient in another ward of the Alfred Hospital, Melbourne, Victoria, Australia. On the basis of phenotypic characteristics, such as biochemical profile and susceptibility pattern, it could not be concluded whether these isolates were distinct from each other or not, which had raised the concern of a common source. To determine the relationship between the strains isolated from these patients, we studied the DNA polymorphism using random amplified polymorphic DNA (RAPD) analysis,[1] which is a variation on a previously described technique.[2,3]

MATERIALS AND METHODS

Bacterial Strains and Biotypes

Routine identification and the determination of antibiotic susceptibility were performed using the automated Vitek system (Biomerieux-Vitek, Hazelwood, MO).

Random Amplified Polymorphic DNA

Random amplified polymorphic DNA was performed with the technique previously described.[1] In brief, 18 μL of a polymerase chain reaction (PCR) mix and cell suspension was amplified in a capillary

thermal cycler using a single decamer primer (5′-ACGTATCTGC-3′). This primer originally was used in PCR typing of *Listeria monocytogenes.*[4] Polymerase chain reaction products were analyzed by electrophoresis in 2% agarose gels and photographed under ultraviolet light.

RESULTS

Between March 1 and March 22, 1995, nine blood culture isolates of *K pneumoniae*, including two central-peripheral venous blood isolate pairs, were obtained from five patients in the 38-bed hematology-oncology ward of Alfred Hospital (range, 1 to 4 isolates per patient). These five patients had either central or peripheral intravascular devices in situ. All of these intravascular devices were being accessed during the period that the bacteremias were appearing. In addition, in the same time period, a bacteremia isolate of *K pneumoniae* was obtained from one patient who was in the geographically distant respiratory ward following cardiac bypass surgery. A further blood culture isolate, initially identified as *K pneumoniae,* was obtained from the hematology-oncology ward on April 12, 1995. There was no increased rate of isolation of *K pneumoniae* from any other ward in the hospital over this time period.

Seven different biotypes, as characterized by the Vitek system, were observed among the 11 isolates. With one exception, the biotypes of the isolates from each patient differed from the biotypes of isolates from other patients by no more than two reactions. All isolates were susceptible to amoxicillin-clavulanate, gentamicin, tobramycin, cefotaxime, ceftazidime, ciprofloxacin, and trimethoprim-sulfamethoxazole, and all were resistant to ampicillin and ticarcillin. All isolates but one were susceptible to cephalothin, and this isolate and three others were resistant to nitrofurantoin.

Three isolates obtained in the first 2 weeks of the cluster were not available for typing. Among the eight isolates from four patients that were available for typing, RAPD analysis revealed four different patterns (Figure). Identical RAPD patterns were observed in the serial isolates (a through c) for each of the two patients (patients 1 and 2) from whom multiple isolates had been obtained. Isolates from separate patients gave distinct patterns. The RAPD pattern of the last isolate (lane 3 in the Figure) was sufficiently different from the others to prompt a re-identification. Reexamination of this isolate, the phenotype of which had differed from the other isolates by more than two biochemical reactions and by its resistance to cephalothin, revealed that it had been misidentified as *K pneumoniae;* its true identification on retesting was *Enterobacter aerogenes.*

Concurrent with the molecular characterization studies of the isolates, an epidemiological investigation of the hematology-oncology ward was initiated during the third week of the epidemic. This investigation failed to identify any source of cross-infection. No further bacteremia isolates of *K pneumoniae* were obtained from this ward in the 3 weeks following the cluster.

DISCUSSION

Klebsiella species are the second most common gram-negative bacteremia isolate at Alfred Hospital. Between January and December 1994, there were 885 positive blood cultures, of which 41 were *Klebsiella* species. In the 60-month period from January 1989 through December 1994, there were 104 bacteremic episodes with *K pneumoniae* among patients of Alfred Hospital. The occurrence of nine blood culture isolates (two of which were duplicate central and peripheral venous isolates) of *K pneumoniae* from the patients of one ward of Alfred Hospital within a 3-week period clearly was unusual, raising the concern that they arose from a single source.

Until recently, epidemiological studies of *K pneumoniae* have been based on the study of phenotypic traits such as biochemical profiles, antibiotic resistance profiles, and serological and phage typing reactions. These techniques are limited in both reproducibility and discriminatory capability. Moreover, bacteriophage and serological typing require the availability of specific reagents that are not readily available outside reference laboratories.

Molecular typing methods are being applied increasingly in the study of nosocomial outbreaks.[5] For example, a study of a cluster of *Enterobacter cloacae* nosocomial isolates using restriction fragment-length polymorphism showed that this technique was more sensitive than phenotyping methods for strain discrimination.[6] Moreover, PCR-based molecular typing techniques similar to RAPD, such as repetitive–element-based PCR,[7] can be sufficiently powerful to demonstrate transmission of more than one strain type within a nosocomial outbreak of *Enterobacter aerogenes* that otherwise appeared homogenous on the basis of phenotypic characterization methods[8] or, conversely, to show clonal identity of epidemiologically related *Klebsiella* isolates that were sufficiently different on biotype criteria to be identified as different species.[9]

Random amplified polymorphic DNA uses an empirically applied random oligonucleotide primer to establish strain-specific DNA fingerprints. Random amplified polymorphic DNA analysis is a rapid and relatively inexpensive method that is easy to perform. It

offers several advantages with respect to speed and simplicity in comparison with other molecular typing methods; for example, ribotyping requires several days, while pulsed-field gel electrophoresis and restriction endonuclease digestion of chromosomal DNA require complex electrophoretic separation techniques and uncommon restriction enzymes. In the series of eight isolates from our hospital, the RAPD technique readily allowed discrimination between the isolates obtained from different patients.

95-CC-212. Address reprint requests to James C. Hurley, MB, BS, PhD, Microbiology, Infectious Diseases and Infection Control, Alfred Group of Hospitals, Prahran, Victoria, 3181, Australia.

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