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Infection Control and Hospital Epidemiology; Jul 2003; 24, 7; Health & Medical Complete
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ANALYSIS OF THREE OUTBREAKS DUE TO *KLEBSIELLA* SPECIES IN A NEONATAL INTENSIVE CARE UNIT

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ABSTRACT

OBJECTIVE: To investigate the clinical, microbiological, and epidemiologic features of three outbreaks caused by *Klebsiella* during 3 years.

SETTING: Neonatal intensive care unit of a university hospital.

PATIENTS: Thirty affected neonates.

METHODS: Data were collected through chart reviews and conversations with physicians. Screening samples were obtained from the staff, the neonates, and the environment. Antibiogram typing and arbitrarily primed polymerase chain reaction–based fingerprinting were used to type the strains.

RESULTS: The first outbreak had 13 *K. pneumoniae* strains isolated. The second outbreak had 10 *K. oxytoca* strains isolated. The third outbreak had 20 *K. pneumoniae* strains isolated. More than half of the patients had low birth weights, were premature, and underwent mechanical ventilation and intravenous catheterization. Approximately three-fourths of the patients died. The isolates tested were completely susceptible to meropenem, cefoxitin, and ciprofloxacin and were resistant to cephalothin. More than half of these strains were resistant to many beta-lactam antibiotics, amikacin, and trimethoprim/sulfamethoxazole. Typing procedures yielded 3 antibiotypes and 3 genotypes among the isolates of the first outbreak, 3 antibiotypes with 1 subtype and 2 genotypes with 1 subtype in the second outbreak, and 2 antibiotypes and 2 genotypes in the third outbreak.

CONCLUSIONS: *Klebsiella* outbreaks mainly affected premature neonates with intravenous catheters, mechanical ventilation, or both. The high mortality rate (76.7%) was notable. Resistance to multiple antibiotics, but mainly to broad-spectrum beta-lactam antibiotics, was observed, particularly in *K. pneumoniae* isolates. Molecular typing indicated that the three outbreaks were not related to one other (*Infect Control Hosp Epidemiol* 2003;24:495-500).

Klebsiella species are pathogens that can cause serious infections such as bacteremia, pneumonia, and urinary tract and soft tissue infections, particularly in immunosuppressed and hospitalized patients. Bacteremia and meningitis are common in pediatric patients, especially those in neonatal intensive care units (NICUs).[1,2] These infections are frequently caused by multidrug-resistant strains and have a high mortality rate.[3] *K. pneumoniae* is the most common pathogen among the *Klebsiella* species[4] and there has been an increase in the incidence of nosocomial infections caused by *K. pneumoniae* strains producing extended-spectrum beta-lactamases.[5-7]

It is important to determine the source of the microorganisms causing an outbreak. To establish the relationship between bacterial isolates, conventional methods such as serotyping, biotyping, antibiotyping, and bacteriophage typing have been replaced in most laboratories by molecular methods such as plasmid analysis, ribotyping, polymerase chain reaction–based typing methods, and pulsed-field gel electrophoresis.[8-10]

In this study, because the three subsequent outbreaks caused by the *Klebsiella* species in the NICU in our hospital in 1999, 2000, and 2001 showed high mortality rates, we sought to define their bacteriologic and clinical features and to determine the clonal relatedness of the 43 *Klebsiella* isolates obtained during them.

METHODS

Patients

This study was performed in Turgut Ozal Medical Center, a 593-bed university hospital with a 16-bed NICU. During a 3-year period, three outbreaks were observed in our hospital. The first outbreak lasted from January 8 to 19, 1999; the second and the third outbreaks lasted from May 25 to June 22, 2000, and from November 11 to December 17, 2001, respectively. The first, second, and third outbreaks included 11, 10, and 9 patients, respectively. The total number of patients admitted to the NICU during each of the three periods was 14, 32, and 28, respectively. Clinical, epidemiologic, and demographic data for each patient were recorded based on chart reviews and conversations with physicians.

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The authors thank the physicians of the neonatal intensive care unit for their help and support.
Presented at the 2nd Molecular and Diagnostic Microbiology Congress; April 21-25, 2002; Antalya, Turkey.

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Screening Studies

During the outbreaks, a total of 163 samples (6 from the skin of the neonates and 44 from the environment in the first outbreak; 7 from the hands of the hospital staff, 4 from the skin of the neonates, and 42 from the environment in the second outbreak; and 10 from the hands of the hospital staff, 7 from the skin of the neonates, 7 from the mouths of the neonates, and 36 from the environment in the third outbreak) were taken to find sources and to help control the spread of *Klebsiella* strains. Environmental sampling involved incubators (44), laryngoscopes (5), connections of the ventilators (11), lipid and total parenteral nutrition solutions (9), stethoscopes (2), disinfectant solutions (6), faucets (3), oxygen jacks (29), equipment trolleys (2), scales (2), urinary catheters (2), aspiration tubings (3), and nebulizers (4).

Hand cultures were performed by pressing fingertips onto blood agar plates. Mouth cultures of neonates, infected or not, were performed during the third outbreak. Cotton swabs moistened with sterile saline were used to swab the skin of neonates. All potentially infected surfaces were also swabbed. Swabs were plated onto eosin–methylene blue agar and blood agar. Approximately 1 to 2 mL of antiseptic solutions and baby foods were inoculated into 10 mL of nutrient broth and incubated for 3 to 4 hours at 35°C in aerobic conditions, then subcultures were performed using blood agar and eosin–methylene blue agar.

Bacterial Isolates

A total of 43 *Klebsiella* isolates were obtained from the patients (35) and the screening samples (8) during the outbreaks. Identification of these isolates to the species level was performed using API 20E (bioMérieux, Marcy-l'Etoile, France).

Antibiotic Susceptibility and Antibiotyping

Antibiotic susceptibility tests were performed using the Kirby–Bauer disk-diffusion method according to criteria of the National Committee for Clinical Laboratory Standards.[11] Amikacin (30 µg), ciprofloxacin (5 µg), trimethoprim/sulfamethoxazole (1.25 µg for the former and 23.75 µg for the latter), meropenem (10 µg), cephalothin (30 µg), cefoxitin (30 µg), ceftazidime (30 µg), ceftriaxone (30 µg), aztreonam (30 µg), and amoxicillin/clavulanic acid (20 µg for the former and 10 µg for the latter) disks (Oxoid, Hampshire, England) were used. The results were recorded as susceptible, resistant, and intermediate. Antibiotyping was performed based on differences among the susceptibility results.[12] Extended-spectrum beta-lactamase production was evaluated using the double disk synergy test.[13]

Arbitrarily Primed Polymerase Chain Reaction (AP-PCR)

The AP-PCR procedure described previously[14] was performed with some modifications. Briefly, each isolate was grown on blood agar overnight at 35°C. Two loops of bacterial colonies were mixed with TE buffer (10 mM of Tris-HCl and 1 mM of EDTA [pH 8.0]) containing proteinase K (500 µg/mL) and 0.5% laroxyl sarcosine, and incubated overnight at 65°C. After phenol-chloroform extraction and ethanol precipitation, the DNA concentration was measured by ultraviolet absorbance at A_{260}. Each AP-PCR reaction mixture (50 µL) contained 100 ng of genomic DNA, 100 pmol of M13 primer (5'-GAG GGT GGC GGT TCT-3'), 2.5 U of Taq DNA polymerase (Promega Corp., Madison, WI), 200 µmol of deoxynucleoside triphosphate mix, 10 mM of Tris-HCl (pH 8.0), 50 mM of KCl, and 2.5 mM of $MgCl_2$. The reaction mixture was amplified with a thermal cycler (PTC-200 Peltier Thermal Cycler; MJ Research Inc., Watertown, MA) according to the following conditions: 2 cycles, each consisting of 5 minutes at 94°C, 5 minutes at 40°C, and 5 minutes at 72°C; and 40 cycles, each consisting of 1 minute at 94°C, 1 minute at 40°C, and 2 minutes at 72°C. Amplification products underwent electrophoresis using 1.5% agarose gel with ethidium bromide and were visualized under ultraviolet illumination.

Dice coefficients of similarity were calculated for every pair of isolates by visual comparison of restriction patterns.[15] Strains with a similarity coefficient of 70% or greater were grouped in a cluster. The isolates with a similarity coefficient equal to or greater than 90% were classified as the same type, those with a similarity coefficient equal to or greater than 70% but less than 90% were classified as a subtype, and those with a similarity coefficient less than 70% were classified as a different genotype.

RESULTS

Patients

A total of 30 patients (14 male and 16 female) were affected during the three outbreaks. All of the patients had a history of empiric antibiotic therapy with a combination of amikacin and ampicillin and intravenous catheterization, and most had received mechanical ventilation before the isolation of the bacteria. In addition, data were available for 10 patients receiving total parenteral nutrition, 2 patients with chest tubes, and 1 patient with cardiac catheterization. Lumbar puncture had been performed in 1 patient, and 2 patients had undergone blood transfusions. Seven patients recovered, but 23 died. Table 1 lists the clinical, demographic, and epidemiologic data of the patients.

Klebsiella *Isolates*

Of the 43 *Klebsiella* strains isolated from the patients and screening cultures, 13, 10, and 20 were obtained during the first, second, and third outbreaks, respectively. The 13 strains during the first outbreak included the isolates from blood (7), tracheal aspirations (4), urine (1), and an incubator (1). During the second outbreak, 9 of the isolates were from blood cultures and 1 was from a tracheal aspirate. The 20 isolates obtained during the third outbreak were from blood (5), tracheal aspirates (6), urine (2), an incubator (1), and mouth cultures (6). Of the 6 mouth isolates, 3 were from infected patients and 3 were from colonized neonates.

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TABLE 1
CLINICAL, EPIDEMIOLOGIC, AND DEMOGRAPHIC DATA OF THE PATIENTS INVOLVED IN THE OUTBREAKS

Outbreak (No. of Patients)	Gender (No. of Patients)	Birth Weight, g (Mean ± SD)	Age at the Date of Positive Culture, d (Mean ± SD)	Length of Time in the NICU Before Positive Culture, d (Mean ± SD)	Major Invasive Procedures (No. of Patients)	Diagnosis at Admission (No. of Patients)
First outbreak (11)	Male (9)	2, 416 ± 1,419	17.5 ± 12.6	15.8 ± 9.5	MV (8)	PR (7), IRDS (3), HB (2),
	Female (2)				IVC (11)	PN (2), CHD (1), C (1), HG (1)
Second outbreak (10)	Male (4)	1,608 ± 848	21.9 ± 13.2	20.6 ± 14.4	MV (8)	PR (8), IRDS (10), C (2),
	Female (6)				IVC (10)	CA (1), PNT (1), HIE (1)
Third outbreak (9)	Male (1)	1,808 ± 996	17.3 ± 16.2	16.3 ± 9.2	MV (8)	PR (7), IRDS (7), CHD (2),
	Female (8)				IVC (9)	HIE (1), MA (1), PNT (1), PN (1)

NICU = neonatal intensive care unit; SD = standard deviation; MV = mechanical ventilation; IVC = intravenous catheterization; PR = prematurity; CHD = congenital heart disease; PN = pneumonia; C = convulsion; HB = hyperbilirubinemia; HG = hypoglycemia; IRDS = infant respiratory distress syndrome; CA = cardiac arrest; PNT = pneumothorax; HIE = hypoxic ischemic encephalopathy; MA = meconium aspiration.

Identification studies showed that 13 *K. pneumoniae*, 10 *K. oxytoca*, and 20 *K. pneumoniae* strains were isolated from the patients and screening samples during the first, second, and third outbreaks, respectively.

Antibiotic Susceptibility

All of the *Klebsiella* isolates tested were susceptible to ciprofloxacin, meropenem, and cefoxitin and were resistant to cephalothin. Rates of resistance to amikacin, trimethoprim/sulfamethoxazole, aztreonam, ceftriaxone, ceftazidime, and amoxicillin/clavulanic acid were 69.8%, 51.2%, 83.7%, 74.4%, 83.7%, and 97.7%, respectively.

Thirty-two (97%) of the 33 *K. pneumioniae* isolates and 2 (20%) of the 10 *K. oxytoca* isolates produced extended-spectrum beta-lactamases.

Subtyping

Antibiogram typing identified three groups among the isolates of the first outbreak. One group consisted of 9 isolates; each of the other two groups had 2 isolates. Of the 10 *K. oxytoca* isolates recovered during the second outbreak, 7 were in one group, 2 were in another group, and 1 showed a unique profile. For the third outbreak, 17 isolates were in one cluster and 3 isolates were in another cluster.

With the use of AP-PCR, the 13 isolates from the first outbreak were genotyped into two groups with one subtype. There were 12 isolates in one group, of which 9 showed the same antibiotype. The second AP-PCR group had 1 isolate. The second outbreak comprised 3 genotypes. Seven isolates were included in one group, 2 in another group, and 1 isolate showed a unique profile. The concordance of the two typing procedures for the strains from the second outbreak was 50%. Two genotypes were found for the third outbreak, consisting of 17 and 3 strains, respectively. There was 100% concordance between antibiotyping and genotyping for this outbreak. The figure shows a sample of the AP-PCR profiles

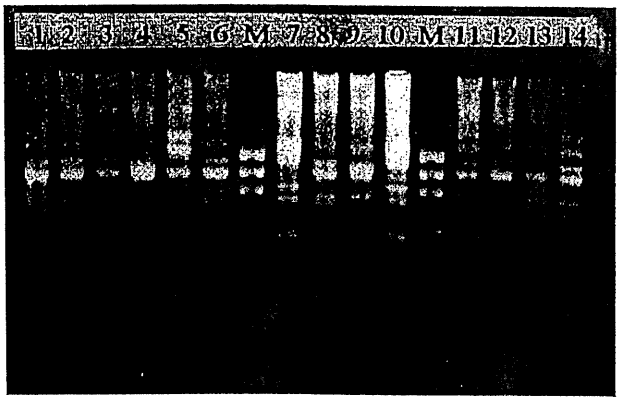


FIGURE. Representative arbitrarily primed polymerase chain reaction patterns of *Klebsiella* species obtained from the three outbreaks. Lines 1 to 6 = the profiles obtained from the isolates of the first outbreak (1, 2, 3, and 6 are in the same cluster; 4 is similar to the cluster profile; and 5 has a unique profile); lines 7 to 10 = the profiles obtained from the isolates of the second outbreak (7 and 10 are in the same cluster and 8 and 9 have a unique profile); and lines 11 to 14 = the profiles obtained from the isolates of the third outbreak (11, 13, and 14 are in the same cluster; and 12 has a unique profile).

obtained from the outbreak strains. Comparison of the genotyping results with those of antibiotyping and epidemiologic grouping can be seen in Table 2.

The clinical isolates were compared with the isolates from the screening samples. The isolates obtained from the blood and the incubator of one patient (A6 in Table 2) during the first outbreak indicated the same genotype and antibiotype, whereas the genotypes and the antibiotypes of the isolates from the blood and the tracheal aspirate of another patient (A2 in Table 2) were distinct. The isolates from different samples of the patients (C7, C8, and C9 in Table 2) and from the screening samples (D1, D2, and D3 in Table 2) of the third outbreak were in the same antibiotype and genotype.

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TABLE 2
COMPARISON OF ANTIBIOTYPES, GENOTYPES, AND EPIDEMIOLOGIC GROUPINGS OF THE OUTBREAK ISOLATES

				Antibiotics Used for Antibiotyping							
Patient*	Material	Species	ESßL	AK	SXT	CAZ	ATM	CRO	AMC	Antibiotype	Genotype
A1	Blood	*Klebsiella pneumoniae*	Positive	R	S	R	R	R	R	1	I
A2	Tracheal aspirate	*K. pneumoniae*	Positive	R	R	R	R	R	R	2	II
	Blood	*K. pneumoniae*	Positive	R	S	R	R	R	R	1	I
A3	Blood	*K. pneumoniae*	Positive	R	R	R	R	R	R	2	I
A4	Blood	*K. pneumoniae*	Positive	R	S	R	R	R	1	I	
A5	Tracheal aspirate	*K. pneumoniae*	Negative	R	S	R	R	R	R	1	I
A6	Blood	*K. pneumoniae*	Positive	R	S	R	R	R	R	1	I
	Incubator	*K. pneumoniae*	Positive	R	S	R	R	R	R	1	I
A7	Urine	*K. pneumoniae*	Positive	R	S	R	R	R	R	1	Ia
A8	Blood	*K. pneumoniae*	Positive	R	S	R	R	R	R	1	I
A9	Tracheal aspirate	*K. pneumoniae*	Positive	R	R	R	R	S	R	3	I
A10	Tracheal aspirate	*K. pneumoniae*	Positive	R	S	R	R	R	R	1	Ia
A11	Blood	*K. pneumoniae*	Positive	R	R	R	R	S	R	3	I
B1	Blood	*K. oxytoca*	Negative	S	S	I	S	I	R	4	III
B2	Blood	*K. oxytoca*	Negative	S	S	I	S	I	R	4	III
B3	Blood	*K. oxytoca*	Negative	S	S	I	S	I	R	4	III
B4	Blood	*K. oxytoca*	Positive	S	S	R	R	I	R	5	IV
B5	Blood	*K. oxytoca*	Negative	S	S	I	S	I	R	4	III
B6	Blood	*K. oxytoca*	Negative	S	S	I	S	I	R	4	III
B7	Blood	*K. oxytoca*	Positive	S	S	I	S	I	R	4	V
B8	Tracheal aspirate	*K. oxytoca*	Negative	S	S	I	S	I	R	4	V
B9	Blood	*K. oxytoca*	Negative	S	S	R	R	I	I	5a	III
B10	Blood	*K. oxytoca*	Negative	S	R	R	R	R	R	6	III
C1	Tracheal aspirate	*K. pneumoniae*	Positive	S	S	R	R	R	R	5b	VI
C2	Tracheal aspirate	*K. pneumoniae*	Positive	S	S	R	R	R	R	5b	VI
C3	Tracheal aspirate	*K. pneumoniae*	Positive	S	S	R	R	R	R	5b	VI
C4	Urine	*K. pneumoniae*	Positive	R	R	R	R	R	R	1	VII
C5	Urine	*K. pneumoniae*	Positive	R	R	R	R	R	R	1	VII
	Blood	*K. pneumoniae*	Positive	R	R	R	R	R	R	1	VII
C6	Tracheal aspirate	*K. pneumoniae*	Positive	R	R	R	R	R	R	1	VII
	Blood	*K. pneumoniae*	Positive	R	R	R	R	R	R	1	VII
C7	Blood	*K. pneumoniae*	Positive	R	R	R	R	R	R	1	VII
	Mouth	*K. pneumoniae*	Positive	R	R	R	R	R	R	1	VII
C8	Tracheal aspirate	*K. pneumoniae*	Positive	R	R	R	R	R	R	1	VII
	Mouth	*K. pneumoniae*	Positive	R	R	R	R	R	R	1	VII
C9	Tracheal aspirate	*K. pneumoniae*	Positive	R	R	R	R	R	R	1	VII
	Blood	*K. pneumoniae*	Positive	R	R	R	R	R	R	1	VII
	Blood	*K. pneumoniae*	Positive	R	R	R	R	R	R	1	VII
	Mouth	*K. pneumoniae*	Positive	R	R	R	R	R	R	1	VII
D1	Mouth	*K. pneumoniae*	Positive	R	R	R	R	R	R	1	VII
	Incubator	*K. pneumoniae*	Positive	R	R	R	R	R	R	1	VII
D2	Mouth	*K. pneumoniae*	Positive	R	R	R	R	R	R	1	VII
D3	Mouth	*K. pneumoniae*	Positive	R	R	R	R	R	R	1	VII

ESßL = extended-spectrum beta-lactamase; R = resistant; S = susceptible; I = intermediate; AK = amikacin; SXT = trimethoprim/sulfamethoxazole; CAZ = ceftazidime; ATM = aztreonam; CRO = ceftriaxone; AMC = amoxicillin/clavulanic acid.
*A = patients in the first outbreak; B = patients in the second outbreak; C = patients in the third outbreak; D = colonized patients.

DISCUSSION

Nosocomial *Klebsiella* infections are mainly caused by *K. pneumoniae*. Less commonly, *K. oxytoca* can also be an etiologic agent for these infections.[1] In agreement with these data, *K. pneumoniae* strains were responsible for two of the three *Klebsiella* outbreaks that occurred in the NICU in our hospital during a 3-year period.

Low birth weight, mechanical ventilation,

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prolonged hospitalization, use of third-generation cephalosporins, and invasive procedures are important risk factors for the emergence of nosocomial infections in intensive care units and for high mortality.[16,17] Although we did not have the opportunity to perform a case–control study analyzing risk factors for *Klebsiella* infections, more than 50% of the neonates affected in these outbreaks had low birth weights, were premature, underwent mechanical ventilation, and had intravenous catheters. However, two of the three neonates colonized with *Klebsiella* in their mouths were premature and had ventilators, but these three neonates recovered without subsequent infection.

Due to a lack of hygienic conditions, *Klebsiella* infections may spread rapidly from medical devices, soap and disinfectants, blood products, and the hands of hospital staff.[1,18] Although no *Klebsiella* species were isolated from the hands of the hospital staff, isolates were obtained from incubators showing the same genotypes and antibiotypes as those obtained from patients during the same outbreak period. In addition, the isolates obtained from the mouths of uninfected neonates during the last outbreak were the outbreak strains. These results confirmed that cross-transmission of *Klebsiella* species occurred among patients, and perhaps from medical devices to patients. This cross-transmission can be controlled by re-emphasizing infection control procedures, especially hand washing practices, and by identifying and eliminating environmental reservoirs. For this purpose, after the first outbreak, the importance of hand washing practices was re-emphasized, the NICU was cleaned with hypochlorite, and the staff were reminded to pay attention to the general hygiene of the rooms and the incubators. Despite these efforts, two subsequent outbreaks occurred possibly due to limited compliance by staff, which may have been due in part to a high frequency of staff rotations in the NICU. Moreover, there was not enough equipment. For instance, sometimes two neonates were put into the same incubator. After the third outbreak, the NICU was revised and new incubators, ventilators, and the other equipment were bought. The nurses attended educational programs on neonatal care and nosocomial infections. An emphasis was placed on keeping the same personnel cleaning the unit to improve consistency. Entry to and exit from the unit were strictly controlled. No other outbreaks have been observed in this unit since the last outbreak in December 2001.

Resistance to expanded-spectrum cephalosporins is reported for most of the outbreak strains of *Klebsiella* species.[3,19,20] Resistance to cefotaxime, ceftazidime, ceftriaxone, and aztreonam is associated with the production of extended-spectrum beta-lactamases.[21,22] In one study performed in our hospital in 1997, this production was reported in 19 (67.9%) of the 28 *Klebsiella* species isolated from the hospitalized patients.[23] In other studies from Turkey, production rates of 49.3% and 34% have been noted in *K. pneumoniae* and other *Klebsiella* species, respectively.[24,25] In the current study, we detected extended-spectrum beta-lactamases in 32 (97%) of the 33 *K. pneumoniae* isolates and in 2 (20%) of the 10 *K. oxytoca* isolates. Isolates that produce extended-spectrum beta-lactamases are resistant to a variety of classes of antibiotics. The isolates tested were completely susceptible to only three antibiotics: meropenem, cefoxitin, and ciprofloxacin. More than half of these strains were resistant to many beta-lactam antibiotics tested, amikacin, and trimethoprim/sulfamethoxazole. Because the outbreaks were caused by multiple resistant strains, their management presented a challenge to physicians. In addition, most of the patients had underlying problems such as respiratory failure and prematurity. Although the overall mortality rate in our NICU was 14.9%, in the current study, more than 75% of the patients with *Klebsiella* infection died. It was concluded that the mortality after *Klebsiella* infection was due to the infection. Neither the mortality rate nor the risk factors recorded differed between the patients infected with strains that were positive for extended-spectrum beta-lactamases and the patients infected with strains that were negative for extended-spectrum beta-lactamases.

Because antibiotyping and PCR-based typing procedures are rapid, inexpensive, and simple, they have frequently been used for the epidemiologic analysis of *Klebsiella* isolates and other microorganisms.[26,27] Random amplified polymorphic DNA was shown to be an effective typing method in another outbreak caused by a multidrug-resistant *K. pneumoniae* in a NICU.[27] In the current study, antibiotyping had 69.2%, 70%, and 85% concordance with the epidemiologic grouping for the first, second, and third outbreaks, respectively. The concordance rates for AP-PCR typing were 92.3% for the first, 70% for the second, and 85% for the third outbreak. There was a 79.1% agreement between antibiotyping and AP-PCR results. Both procedures yielded the same types among the isolates from different samples of the same patients, except for two isolates of one patient.

As in other studies, the *Klebsiella* outbreaks in our pediatric ward mainly affected premature neonates with ventilators. Multiple antibiotic resistance to extended-spectrum beta-lactam antibiotics was observed, particularly among the *K. pneumoniae* isolates. The three outbreaks were found to be unrelated to each other based on the analysis of antibiotypes and genotypes of their isolated strains.

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VOLUME 24, NUMBER 7 JULY 2003

The Official Journal of The Society for Healthcare Epidemiology of America

PUBLISHED FOR THE SOCIETY BY SLACK INCORPORATED

INFECTION CONTROL AND HOSPITAL EPIDEMIOLOGY™

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