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Study of an Outbreak of Cefoxitin-Resistant *Klebsiella pneumoniae* in a General Hospital

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During a 3-month period, six *Klebsiella pneumoniae* isolates resistant to cefoxitin and penicillin-inhibitor combinations were derived from patients in the intensive care unit of a hospital in Athens, Greece. Enterobacterial repetitive intergenic consensus PCR and pulsed-field gel electrophoresis provided evidence of the clonal origin of the isolates. Conventional techniques and ribotyping were inadequate in proving that the isolates were related. Resistance was due to a plasmidic class C β-lactamase.

Klebsiella pneumoniae is often implicated in hospital infections, and many outbreaks of strains producing extended-spectrum β-lactamases (ESBL) have been reported (2, 6, 16). The most common ESBL found in *K. pneumoniae* in Greek hospitals is an SHV-5-type β-lactamase that confers resistance to ceftazidime and aztreonam but not to cefoxitin and penicillin-inhibitor combinations (8, 15). Recent reports have also described the isolation of *K. pneumoniae* strains expressing plasmid-mediated AmpC-type cephalosporinases (1, 5, 12, 14). These strains display reduced susceptibility to virtually all β-lactams, including cefoxitin and penicillin-inhibitor combinations, and have been sporadically isolated in hospitals in Athens, Greece (1, 5, 14). We observed recently that cefoxitin-resistant (FOX^r) *K. pneumoniae* isolates were repeatedly recovered from clinical material in the intensive care unit (ICU) of a general hospital in Athens. In this work, we attempted to type these strains with the aid of conventional and molecular techniques.

From November 1994 to February 1995, six nonrepetitive (one per patient) FOX^r *K. pneumoniae* strains (Kp1 to Kp6) were isolated from hospitalized patients in the ICU of Evagelismos Hospital. Five isolates were from blood cultures, and one was from sputum. Two cefoxitin-susceptible (FOX^s) *K. pneumoniae* clinical isolates from Evagelismos Hospital (Kp7 and Kp8) and two FOX^r strains (Kp9 and Kp10) isolated in other Athens hospitals during the same period were used as controls. Four β-lactam-susceptible *K. pneumoniae* strains isolated from the personnel (KpPH-1 and KpPH-2) and from the environment (KpEV-1 and KpEV-2) of the ICU during the attempt to trace the potential reservoir of the FOX^r isolates were also included in the study. The strains were identified by the API 20E (API-BioMerieux). MICs of β-lactams were determined by the E test (Biodisk). Susceptibilities to aminoglycosides, fluorinated quinolones, and trimethoprim-sulfamethoxazole were evaluated by antibiograms. Capsular typing was performed by countercurrent immunoelectrophoresis (11) with 72 antisera. Ribotyping was performed with DNA extracted with guanidium thiocyanate (4) and digested with *Hin*dIII and *Eco*RI. The probe used was from rRNA of *Escherichia coli* (Boehringer) after nonradioactive labeling with random hexanucleotide primers (Boehringer), reverse transcriptase (Gibco-BRL), and biotin-16-dUTP. The strains were also typed by enterobacterial repetitive intergenic consensus (ERIC) PCR with the ERIC-2 primer (5′ AAGTAAGTGAC TGGGGTGAGCG 3′) (7). For chromosomal analysis by pulsed-field gel electrophoresis (PFGE), genomic DNA was prepared in agarose plugs and digested with *Xba*I. DNA fragments were separated in agarose gels in a contour-clamped homogeneous electric field apparatus (Bio-Rad) (6). For isoelectric focusing (IEF) of β-lactamases, clarified ultrasonic extracts of bacterial broth cultures were run in polyacrylamide gels containing ampholytes (pH range, 3.5 to 9.5) (Pharmacia-LKB). The β-lactamase bands were visualized with nitrocefin (Oxoid).

Five of the FOX^r isolates (Kp1 to Kp5) displayed similar resistance phenotypes. They were resistant to the tested β-lactams. IEF showed that these isolates expressed two β-lactamases with isoelectric points (pIs) of 9.1 and 8.2. In three of them, an enzyme focused at a pI of 5.4 was also present. Isolate Kp6 was resistant to cefoxitin and amoxycillin-clavulanate and displayed reduced susceptibility to ceftazidime, aztreonam, and cefotaxime; it produced one β-lactamase with a pI of 9.1 (Table 1). Conjugation experiments with *E. coli* 26R793 (Rif^r *lac*) as the recipient, determination of the antibiotic susceptibilities of the transconjugant clones, and IEF showed that resistance to cefoxitin was due to the expression of a plasmid-mediated β-lactamase with a pI of 9.1 (data not shown). The enzyme, on the basis of its pI and substrate and inhibition profiles and the resistance phenotype conferred, appears to be similar to the previously described plasmid-mediated cephalosporinases LAT-1 and LAT-2 derived from *Citrobacter freundii* (5, 13). The cephalosporinase-encoding plasmids from all six *K. pneumoniae* FOX^r isolates from the ICU had the same molecular size (8.3 kb) and gave restriction patterns indistinguishable from those of the LAT-encoding plasmids (5). The enzyme detected here was unable to confer high-level resistance to oxyiminocephalosporins and aztreonam. Apparently, the simultaneous presence of an enzyme with a pI of 8.2, presumably an SHV-5 type that is frequently encountered in enterobacteria from Greek hospitals (8, 15), resulted in high-level resistance to oxyimino-β-lactams.

All six FOX^r outbreak isolates cross-reacted with capsular antisera to K7 and K61. Four of these isolates had the same API 20E biotype, while the other two differed by a single reaction each. The control isolates reacted with different anti-

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TABLE 1. Sources, biochemical characteristics, serotypes, β-lactamase contents, and β-lactam susceptibilities of *K. pneumoniae* isolates examined in this study

Isolate	Source	Presence of following biotype[a]:				Serotype	β-Lactamase pI(s)	E-test MIC(s) (μg/ml)[b]				
		ADH	LDC	VP	GLU			AMC	FOX	CAZ	ATM	CTX
Kp1	Blood	−	+	+	+	K7/K61	5.4, 8.2, 9.1	48, 24	256	128	128	64
Kp2	Blood	−	+	+	+	K7/K61	5.4, 8.2, 9.1	48, 24	128	128	64	64
Kp3	Blood	−	+	+	−	K7/K61	5.4, 8.2, 9.1	48, 24	256	128	128	64
Kp4	Sputum	−	+	+	+	K7/K61	8.2, 9.1	32, 16	128	128	64	32
Kp5	Blood	−	+	+	+	K7/K61	8.2, 9.1	32, 16	64	128	64	32
Kp6	Blood	−	−	+	+	K7/K61	9.1	32, 16	64	16	8	8
Kp7	Urine	+	+	+	+	K27/K46	5.4, 8.2	16, 8	4	64	64	4
Kp8	Blood	−	+	+	+	K61	8.2	6, 3	4	128	64	4
Kp9	Pus	−	+	−	+	K11/K21/K26	8.2, 9.4	48, 24	128	128	128	32
Kp10	Blood	+	+	+	+	K13	9.1	40, 20	64	16	16	16
KpPH-1	Personnel	+	+	+	+	K8		2, 1	2	0.25	0.12	0.06
KpPH-2	Personnel	−	+	−	+	K11/K21		2, 1	2	0.12	0.12	0.12
KpEV-1	Environment	+	+	+	+	K61	5.4	4, 2	4	0.12	0.06	0.06
KpEV-2	Environment	−	+	+	+	K3/K7/K68		2, 1	2	0.25	0.12	0.06

[a] ADH, arginine dihydrolase; LDC, lysine decarboxylase; VP, Voges-Proskauer; GLU, glucose utilization.
[b] AMC, amoxycillin-clavulanate; FOX, cefoxitin; CAZ, ceftazidime; ATM, aztreonam; CTX, cefotaxime.

sera (Table 1). The rDNA fingerprints of the six outbreak isolates were similar; however, only a few hybridization bands were detected and these showed no marked divergence from those of the controls. More distinct banding patterns were observed with ERIC PCR. The FOX^r outbreak strains gave similar ERIC PCR patterns which were different from those of the control strains (Fig. 1). Analogous results were obtained with PFGE. All but one FOX^r outbreak strain displayed similar patterns that were readily distinguishable from those of the control strains. In one isolate (Kp6), a DNA band present in the remaining five FOX^r isolates was missing (Fig. 2).

The appearance of isolates with such an uncommon resistance phenotype in a hospital ward suggested an outbreak. There were, however, reservations based on the following facts. (i) Similar resistance phenotypes have been observed among ESBL-producing *K. pneumoniae* strains isolated in Athens hospitals, but typing showed that they were distinct (8). (ii) The LAT-1 and LAT-2-encoding plasmids, although not self-transferable, can be mobilized by enterobacterial conjugative plasmids (5, 14). Therefore, dissemination of a single R plasmid in distinct *K. pneumoniae* clones could not be ruled out. (iii) Three different biotypes were observed. (iv) One of the six FOX^r isolates had a different susceptibility pattern. (v) Differences in β-lactamase content were also observed. However, the results obtained by ERIC PCR and PFGE showed clearly that there was an outbreak of FOX^r *K. pneumoniae* in the ICU of Evagelismos Hospital. We have not been able to determine the original source of the outbreak strain or the mode of its transmission. The outbreak strain seemed to be confined to the ICU despite the usual movement of patients from the unit to other wards. It was hypothesized that the strain was carried by a stable vector such as a contaminated device or an ICU staff member. In the following sampling, FOX^r *K. pneumoniae* strains were not detected. The four β-lactam-susceptible strains isolated from finger imprints of the staff members and

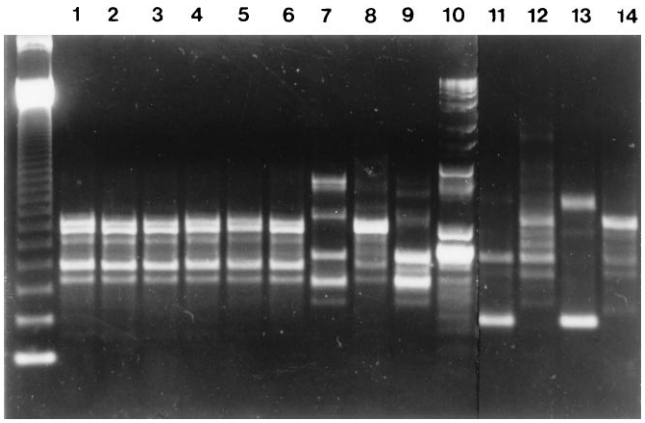

FIG. 1. ERIC PCR patterns of the *K. pneumoniae* isolates studied. Lanes: 1 to 6, outbreak isolates Kp1 to Kp6, respectively; 7 to 14, control strains Kp7, Kp8, Kp9, Kp10, KpPH-1, KpPH-2, KpEV-1, and KpEV-2, respectively. Molecular size markers (123-bp DNA ladder from GIBCO-Bethesda Research Laboratories) are on the left.

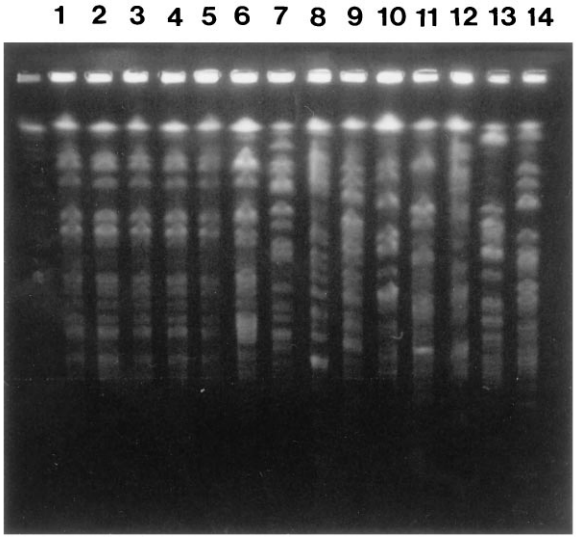

FIG. 2. RFLPs of cefoxitin-resistant *K. pneumoniae* Kp1 to Kp6 (lanes 1 to 6, respectively), control isolates Kp7 to Kp10 (lanes 7 to 10, respectively), and isolates KpPH-1, KpPH-2, KpEV-1, and KpEV-2 (lanes 11 to 14, respectively). DNA preparations were cut with *Xba*I and analyzed by contour-clamped homogeneous electric field–PFGE as described in the text. Low-molecular-weight markers (*Saccharomyces cerevisiae*; Bio-Rad) are on the left.

the ventilator tube T junction were different from the outbreak isolates (Table 1 and Fig. 1).

Resistance of *K. pneumoniae* to cefoxitin is considered uncommon, and susceptibility to the antibiotic has been proposed as an additional means to distinguish *K. pneumoniae* from enterobacters that produce inducible cephalosporinases (3). However, FOX^r *K. pneumoniae* strains harboring plasmids coding for AmpC-type β-lactamases are established in our hospitals. Acquisition of such plasmids expands the resistance spectrum of *K. pneumoniae* towards cephamycins and, most importantly, to β-lactam-inhibitor combinations. The increasing use of the latter drugs may assist the selection of such strains.

K. pneumoniae is the cause of at least 14% of the gram-negative infections in Greek hospitals (9). Thus, there is a need for a simple, reliable, and low-cost typing method. Investigations of the nosocomial epidemiology of bacteria have long been hampered by the relatively low discriminatory capacity of the common phenotypic traits. Serotyping may be a useful means for the preliminary discrimination of *K. pneumoniae* strains, but K antisera are not commercially available. It is of interest that the majority of the *K. pneumoniae* strains isolated in Greek hospitals either cross-react with two or more K antisera or are nontypeable. A possible explanation is that the strains belong to "novel" serotypes. Alternatively, the cross-reactions represent surface changes that may accompany pleiotropic mutations conferring antibiotic resistance. Additional phage typing enhances the ability to distinguish the isolates, but the method is tedious and its reproducibility is low. Conventional techniques also include biotyping and determination of susceptibility patterns. Both techniques were unsuccessful in proving a relationship between the isolates examined. Biochemical characteristics and susceptibility patterns may vary between isolates of the same strain, particularly in a hospital setting. Random mutations, selected by antibiotics, might affect one or more biochemical traits, while the acquisition or loss of R plasmids can change the susceptibility patterns.

Molecular techniques that are based on inherently stable bacterial characteristics usually provide more detailed and accurate typing results. However, the discriminatory power of ribotyping, as applied here, appeared inadequate. rDNA genes are conserved within enterobacterial species, and distinct strains may produce similar ribopatterns (10). The patterns produced by ERIC PCR and those from PFGE showed clearly that all six FOX^r isolates were derived from a single clone. The PCR patterns were identical. The PFGE patterns were similar for strains Kp1 to Kp5. Notably, a high-molecular-weight band was consistently absent from the ladder pattern of strain Kp6. This might be due to random genetic rearrangements or to the lack of a conjugative R plasmid present in isolates Kp1 to Kp5. PFGE is the reference method for typing of strains of several species, but it is time consuming and requires expensive hardware. ERIC PCR, as applied here, was rapid, convenient, and able to reveal the clonal origin of the outbreak isolates.

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