MAJOR ARTICLE

Risk Factors for Increasing Multidrug Resistance among Extended-Spectrum β-Lactamase–Producing *Escherichia coli* and *Klebsiella* Species

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Background. The importance of infections due to extended-spectrum β-lactamase–producing *Escherichia coli* and *Klebsiella* species (ESBL-EK) has been increasingly recognized in recent years. ESBL-EK infections are of clinical concern, because few antimicrobials are available as therapeutic options. Increased reliance on carbapenems has led to increasing carbapenem resistance. Efforts to maintain current therapeutic options for ESBL-EK infections are essential.

Methods. We conducted a case-control study to identify risk factors for multidrug resistance (MDR) among ESBL-EK. All patients at our institution who had an inpatient clinical culture result positive for an ESBL-EK during the period of 1 June 1997 through 31 December 2002 were eligible for inclusion. An MDR ESBL-EK was defined as ESBL-EK demonstrating resistance to trimethoprim-sulfamethoxazole, aminoglycosides, and quinolones. All available ESBL-EK isolates were characterized by pulsed-field gel electrophoresis (PFGE).

Results. Of 361 total ESBL-EK isolates, 68 (18.8%) were MDR. During the study period, the prevalence of MDR among ESBL-EK isolates increased from 12.5% to 26.9%. The only independent risk factor for MDR ESBL-EK was the infecting organism (i.e., *Klebsiella pneumoniae*; adjusted odds ratio, 11.7; 95% confidence interval, 4.77–28.51; $P < .001$). Prior antibiotic use was not independently associated with MDR ESBL-EK. PFGE patterns from *K. pneumoniae* isolates indicated close genetic relatedness among a substantial proportion of isolates.

Conclusions. The emergence of MDR among ESBL-EK has important implications for the future ability to treat these infections. The strong association between the species of infecting organism and MDR suggests that the epidemiology in *K. pneumoniae* may be unique. PFGE results suggest that horizontal spread is important in the emergence of MDR ESBL-EK.

The oxyimino β-lactams were introduced in 1981. By 1983, extended-spectrum β-lactamase (ESBL)–producing organisms had already been isolated in Germany [1] and were reported in the United States by 1989 [2, 3], with outbreaks of infections in the United States noted soon thereafter [4, 5]. In recent years, the importance of such ESBL-mediated infections has been increasingly recognized [6–8]. The association of ESBL-producing infections with both negative clinical outcomes and increased cost is of great concern [9, 10].

Resistance to additional classes of antibiotics has been noted among ESBL-producing *Escherichia coli* and *Klebsiella* species (ESBL-EK) [7, 11, 12]. With resistance to each additional class of antibiotics, ESBL-EK infections become a greater therapeutic challenge. Reliance on carbapenems has increased, because they are the only class of agents to which ESBL-EK remain almost uniformly susceptible. However, empirical treatment of suspected ESBL-EK infections with carbapenems has been associated with significant increases in carbapenem resistance in other organisms (e.g., *Pseudomonas aeruginosa* and *Acinetobacter calcoaceticus*) [13–15]. Ad-

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ditionally, recently reported carbapenem-resistant ESBL-EK isolates are of paramount concern because of the scarcity of effective therapies for infections with such organisms [16, 17].

Multidrug-resistant (MDR) ESBL-EK isolates (i.e., those resistant to multiple other antibiotics or antibiotic classes in addition to the oxyimino β-lactams) pose significant therapeutic challenges, even greater than those of ESBL-EK. Thus, it is essential to characterize the risk factors associated with MDR ESBL-EK so that effective strategies can be introduced to curb their emergence. To our knowledge, this issue has not previously been investigated. We conducted this study to identify longitudinal trends in the emergence of MDR among ESBL-EK at our institution and to characterize the clinical and molecular epidemiology of such infections.

METHODS

This study was conducted at 2 hospitals in the University of Pennsylvania Health system: the Hospital of the University of Pennsylvania (Philadelphia), a 625-bed academic tertiary care medical center, and Presbyterian Medical Center (Philadelphia), a 344-bed urban community hospital. Study subjects were identified through records of the Clinical Microbiological Laboratory at the Hospital of the University of Pennsylvania, which performs cultures for bacteria for all clinical specimens obtained at these institutions. Records from the Department of Infection Control were also reviewed to ensure that all eligible subjects were identified. All adult patients who had a positive result of inpatient clinical culture for an ESBL-EK during the period of 1 June 1997 through 31 December 2002 were eligible for inclusion. Patients could be included >1 time if they had multiple positive results of cultures for ESBL-EK during the study period. The earliest ESBL-EK isolate identified was reviewed and included, with each subsequent episode included only if it occurred >30 days after the prior isolate.

To assess longitudinal trends in the susceptibility of ESBL-EK to other antibiotics and antibiotic classes, the resistance profiles of all eligible ESBL-EK isolates were determined. An MDR ESBL-EK was defined as an ESBL-EK that demonstrated resistance to all the following 3 antibiotics or antibiotic classes: trimethoprim-sulfamethoxazole, aminoglycosides (i.e., amikacin, netilmicin, gentamicin, and tobramycin), and fluoroquinolones (i.e., ciprofloxacin, ofloxacin, and levofloxacin). The prevalence of ESBL-EK with resistance to 1 other antibiotic class (e.g., fluoroquinolones and aminoglycosides) was also calculated for each 1-year time interval. In addition, the annual prevalence of MDR ESBL-EK was assessed.

To identify risk factors for MDR ESBL-EK, we conducted a case-control study. The study question we sought to answer was "In patients who develop ESBL infections, what are the risk factors for that ESBL infection demonstrating multidrug resistance?" On the basis of this study question, all patients with a culture positive for an MDR ESBL-EK were designated as case patients; control patients were those patients with an ESBL-EK isolate that did not meet criteria for multidrug resistance [18]. As noted, every ESBL-EK isolate identified by the Clinical Microbiological Laboratory or Infection Control was eligible for inclusion, and all eligible case patients and control patients were included, by use of the eligibility criteria noted above. Of note, because we focused on an adult population, the few ESBL-EK isolates ($n = 6$) recovered from neonates were not eligible.

Potential risk factors for MDR ESBL-EK were ascertained by review of the inpatient medical record. Data obtained included age, sex, race, hospital location at the time of infection, duration of hospital stay and intensive care unit stay (in days) before infection, and severity of illness, as calculated by the APACHE II score [19]. The presence of a central venous catheter, urinary catheter, or mechanical ventilation was documented, as was the site of infection, the species of infecting organism, and its antibiotic susceptibility profile. Finally, all antimicrobial therapy administered in the 30 days before the positive culture result was ascertained.

The presence of the following comorbid conditions was documented: hepatic dysfunction, malignancy, diabetes mellitus, renal insufficiency (indicated by a creatinine level of >2.0 mg/dL or the requirement of dialysis), HIV infection, neutropenia, corticosteroid use, prior organ transplantation, use of an immunosuppressive agent in the 30 days before infection, surgical procedure or trauma in the 30 days before infection, paralysis, and the presence of decubitus ulcers.

Nosocomial acquisition of infection was defined as follows: infection that occurred >48 h after admission to the hospital or infection that occurred ⩽48 h after admission to the hospital if that patient had been hospitalized within the 30 days before admission or if the patient was transferred from an outside hospital or nursing home. Also noted was whether patients fulfilled the criteria for infection, as delineated by the Centers for Disease Control and Prevention [20].

Microbiological methods. Susceptibilities to all antimicrobial agents were determined and interpreted according to criteria of the NCCLS by means of either a semiautomated system (MicroScan WalkAway System, NC16 panel, Dade Behring; bioMérieux) or disk diffusion susceptibility testing. During each time period, we used the time period–relevant NCCLS guideline for detecting and confirming *E. coli, Klebsiella pneumoniae,* or *Klebsiella oxytoca* isolates producing an ESBL [21–23].

Available isolates, mostly from 1999 and 2000, were evaluated for genetic relatedness by PFGE, which was done according to Gautom [24] with *Xba*I digestion of genomic DNA (New England Biolabs) with slight modifications, as described elsewhere [12]. A CHEF Mapper apparatus (Bio-Rad) was used for electrophoresis with an initial switch time of 3 s and final switch

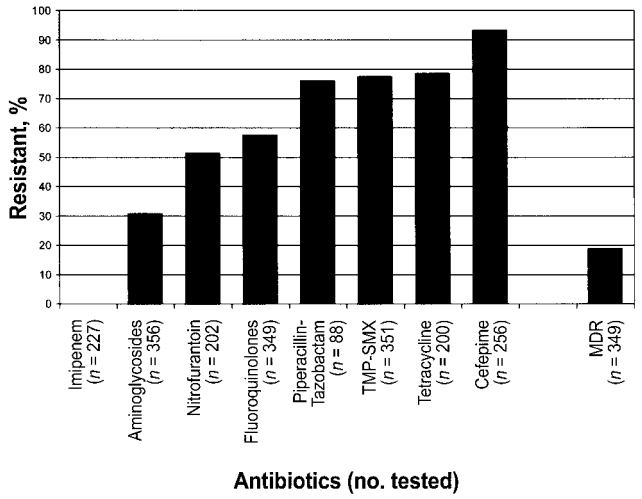

Figure 1. Antimicrobial susceptibilities of extended-spectrum β-lactamase–producing *Escherichia coli* and *Klebsiella* species isolates (1997–2002). MDR, multidrug resistance; TMP-SMX, trimethoprim-sulfamethoxazole.

time of 40 s, with a total run time of 21 h. Each gel contained 2 standard lanes of λ Ladder PFGE Marker (New England Biolabs). Band patterns were compared with Fingerprinting II Software, version 3.0 (Bio-Rad). Dendrograms were generated by the unweighted pair group method with arithmetic averages. Position tolerances were set at 1.0%; Dice coefficient was used for band similarity measurements. Fingerprinting data were interpreted in accordance with established guidelines [25].

Statistical methods. To determine longitudinal trends in susceptibilities of ESBL-EK to various antimicrobials, we calculated the prevalence of ESBL-EK isolates that were resistant to other classes of antibiotics for each year of the study period. We assessed resistance to aminoglycosides, cefepime, imipenem, nitrofurantoin, piperacillin-tazobactam, quinolones, tetracycline, and trimethoprim-sulfamethoxazole. We also calculated the annual proportion of ESBL-EK isolates that were MDR. To evaluate the trend in the proportion of positive tests over time, the Cochran-Armitage trend test (χ^2 test for trend) was done [26].

Bivariable analysis was conducted to determine the association between potential risk factors and MDR ESBL-EK. Categorical variables were compared by Fisher's exact test. An OR and 95% CI were calculated to evaluate the strength of any association. Continuous variables were compared by Student's *t* test or the Wilcoxon rank sum test, depending on the validity of the normality assumption [27].

Multivariable analysis was done by multiple logistic regression [28]. Building of the multivariable model began with inclusion of key variables based on a priori hypotheses (i.e., prior antibiotic use). All variables with $P < .20$ on bivariable analysis were also considered for inclusion in a multivariable explanatory model [29]. Finally, variables were considered for inclusion in the model if they were noted to be involved in confounding or interaction on stratified analysis. The interaction between risk factor variables was also investigated. A 2-tailed P value of <.05 was considered to be statistically significant. All statistical calculations were done with standard programs in STATA, version 8.0 (Stata).

RESULTS

During the study period, 547 inpatient ESBL-EK isolates were identified, of which 361 fulfilled all inclusion criteria and were included in the study. Of these 361 included isolates, 345 (95.6%) had the full medical record available for review, and the remainder were assessed via review of partial medical records and other data sources (e.g., the clinical microbiology laboratory report and infection-control records).

Of the 361 ESBL-EK isolates, 151 (41.8%) were *E. coli*, 183 (50.7%) were *K. pneumoniae*, and 21 (5.8%) were *K. oxytoca*. Additionally, 1 isolate was polymicrobial and had both species

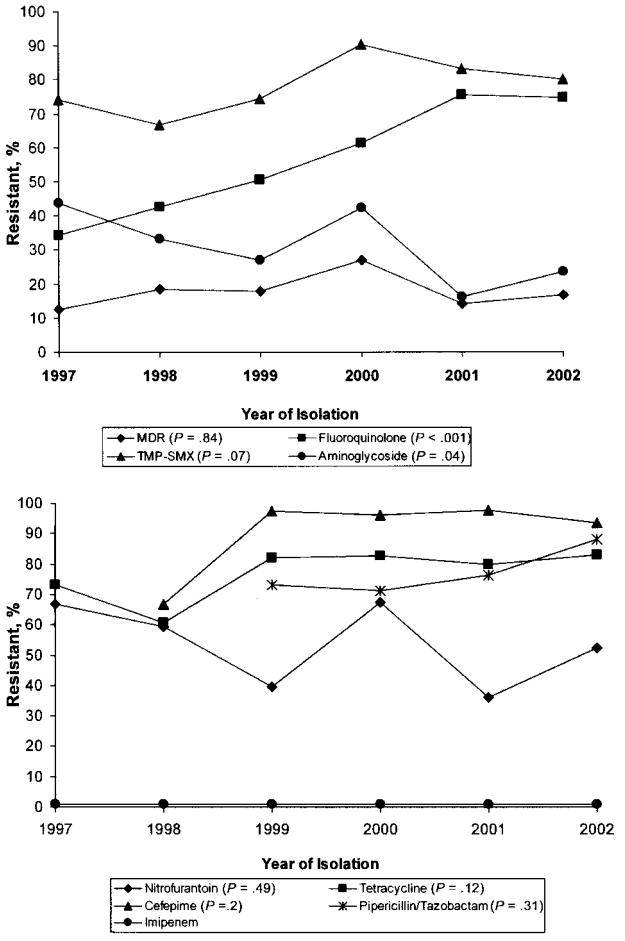

Figure 2. Longitudinal trends in resistance among extended-spectrum β-lactamase–producing *Escherichia coli* and *Klebsiella* species (1997–2002). *Top,* Antibiotics and antibiotic classes that defined multidrug resistance (MDR). *Bottom,* Other antibiotics for which resistance was assessed. TMP-SMX, trimethoprim-sulfamethoxazole.

Table 1. Characteristics of patients who were infected with multidrug-resistant extended-spectrum β-lactamase–producing *Escherichia coli* and *Klebsiella* species (ESBL-EK; case patients) and of patients infected with ESBL-EK isolates were not multidrug-resistant (control patients).

Variable	Case patients	Control patients	OR (95% CI)	P[a]
Age, mean years (range)	62 (19–88)	64 (17–98)	…	.53
Male sex	30/68 (44.1)	137/293 (46.8)	0.90 (0.53–1.52)	.69
African American race	26/47 (55.3)	121/220 (55.0)	1.01 (0.54–1.90)	.97
Hospitalized at HUP	55/68 (80.9)	215/293 (73.4)	1.53 (0.80–2.94)	.20
Nursing home residence	16/63 (25.4)	82/275 (29.8)	0.80 (0.43–1.49)	.49
Hospitalized in past 30 days	38/65 (58.5)	142/272 (52.2)	1.29 (0.75–2.22)	.36
Transferred from outside hospital	18/63 (28.6)	70/277 (25.3)	1.18 (0.65–2.17)	.59
APACHE II score, mean (range)	9.5 (0–25)	10 (0–29)	…	.90
Duration of hospitalization, mean days (range)[b]	10 (0–105)	5 (0–288)	…	.20
Infection[c]	52/58 (89.7)	242/258 (93.8)	0.57 (0.22–1.49)	.26
Nosocomial infection	63/65 (96.9)	252/283 (89.0)	3.88 (0.94–34.19)	.06
Diarrhea	6/60 (10)	19/273 (7.0)	1.49 (0.58–3.79)	.42
Catheter present				
Central venous catheter	40/61 (65.6)	120/273 (44.0)	2.43 (1.37–4.31)	.003
Urinary	48/62 (77.4)	191/276 (69.2)	1.53 (0.80–2.89)	.22
Mechanical ventilation	21/63 (33.3)	60/276 (21.7)	1.8 (0.99–3.25)	.07

NOTE. Data are *n/N* (%), unless otherwise indicated. HUP, Hospital of the University of Pennsylvania (Philadelphia).

[a] By Fisher's exact test for categorical variables and Wilcoxon rank sum test for continuous variables.

[b] No. of days from hospital admission until infection with ESBL-EK.

[c] As opposed to colonization.

of *Klebsiella* present. Five (1.4%) isolates did not have the species of infecting organism documented in the records available.

These 361 ESBL-EK isolates demonstrated variable resistance to other antibiotics and antibiotic classes (figure 1). Although 68 of the ESBL-EK (18.8%) fulfilled criteria for MDR, no ESBL isolates were resistant to imipenem. Although not included in our definition of MDR ESBL-EK, there was a high prevalence of resistance to cefepime and piperacillin-tazobactam. Furthermore, there was a 91.6% concordance between susceptibility results for these 2 agents. The prevalence of resistance to other agents, including the MDR phenotype, was similar for isolates that were resistant or susceptible to cefepime and piperacillin-tazobactam.

A significant increase in the prevalence of fluoroquinolone resistance ($P < .001$) was evident among the ESBL-EK over the 5.5-year study period (figure 2). In addition, a borderline significant increase in the prevalence of trimethoprim-sulfamethoxazole resistance was also observed ($P = .07$). Finally, a significant decrease in the prevalence of aminoglycoside resistance among ESBL-EK occurred ($P = .04$). Although yearly prevalence of MDR ESBL-EK increased from 12.5% to as high as 26.9%, this trend was not statistically significant.

To identify risk factors for MDR ESBL-EK, we compared the 68 case patients with MDR ESBL-EK infection with the 293 control patients with ESBL-EK infection (table 1). With regard to baseline demographic characteristics, there were no significant differences between case patients and control patients. The presence of a central venous catheter was significantly more common in case patients. Case patients were more likely than control patients to have been located in an intensive care unit at the time of infection (50.8% vs. 34.4%) (OR, 1.97; 95% CI, 1.14–3.38; $P = .02$). When comorbidities were compared between case patients and control patients, no significant differences existed. With regard to the anatomic site of infection,

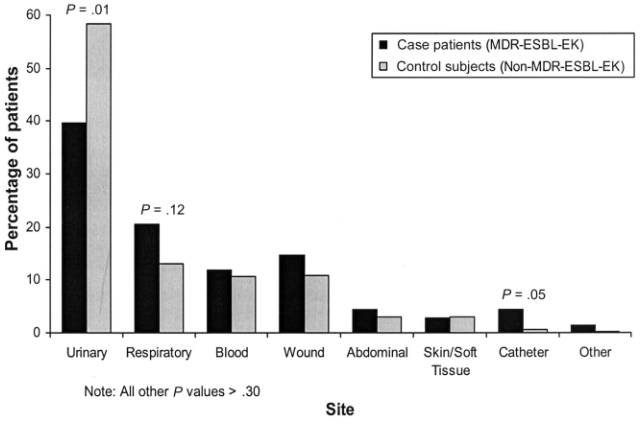

Figure 3. Sites of infection with extended-spectrum β-lactamase–producing *Escherichia coli* and *Klebsiella* species (MDR ESBL-EK).

Table 2. Prior antimicrobial exposure of patients infected with multidrug-resistant extended-spectrum β-lactamase–producing *Escherichia coli* and *Klebsiella* species (ESBL-EK; case patients) and patients infected with ESBL-EK isolates were not multidrug-resistant (control patients).

Variable	Case patients (n = 68)	Control patients (n = 293)	OR (95% CI)	P[a]
General				
Prior use of any antibiotic	50 (73.5)	199 (67.9)	1.31 (0.73–2.36)	.37
Total prior antibiotics, median no. (range)	3 (0–8)	2 (0–9)	…	.13
Total antibiotic-days,[b] median (range)	10.5 (0–77)	8 (0–98)	…	.15
Specific				
Fluoroquinolone	27 (39.7)	77 (26.3)	1.85 (1.07–3.19)	.03
Trimethoprim-sulfamethoxazole	9 (13.2)	57 (19.5)	0.63 (0.30–1.33)	.30
Aminoglycoside	22 (32.4)	74 (25.3)	1.42 (0.80–2.50)	.29
Third-generation cephalosporin	5 (7.4)	17 (5.8)	1.28 (0.48–3.50)	.58
β-Lactam/β-lactamase inhibitor[c]	13 (19.1)	49 (16.7)	1.18 (0.60–2.30)	.60
Antianaerobic agent[d]	33 (48.5)	127 (43.3)	1.66 (0.98–2.81)	.07

NOTE. Data are no. (%) of subject, unless otherwise indicated.

[a] By Fisher's exact test for categorical variables and Wilcoxon rank sum test for continuous variables.

[b] Twenty-six patients who received antibiotic therapy for an unknown duration were excluded.

[c] Amoxicillin/clavulanate, ampicillin/sulbactam, and piperacillin-tazobactam.

[d] Amoxicillin/clavulanate, ampicillin/sulbactam, piperacillin-tazobactam, imipenem, meropenem, metronidazole, clindamycin, chloramphenicol, and trovafloxacin.

case patients were significantly less likely to have a urinary infection (figure 3).

Ninety percent of case patients were infected with *K. pneumoniae*, compared with only 42.7% of control patients (OR, 11.69; 95% CI, 5.26–25.92; $P < .001$). Case patients and control patients did not differ with regard to whether the infection was polymicrobial (30.9% vs. 32.0%) (OR, 0.95; 95% CI, 0.54–1.67; $P = .89$).

Case patients and control patients did not differ significantly in overall antibiotic use prior to infection regardless of whether antibiotic use was expressed as "total antibiotic days" or "total number of antibiotics" (table 2). In terms of specific antibiotics taken, case-patients were significantly more likely to have received fluoroquinolone therapy in the 30 days before infection.

After multivariable analysis, the only independent risk factor for MDR-ESBL infection was the infecting pathogen (i.e., *K. pneumoniae*). There was also a borderline significant association between the presence of a central venous catheter and MDR ESBL-EK infection (table 3). No association was seen between prior antibiotic exposure (in days of antibiotic therapy) and the presence of MDR infection. Similarly, when including the variables for use of specific agents (e.g., fluoroquinolones and aminoglycosides) rather than the variable for overall antibiotic use (i.e., total duration of antibiotic use in days), no association existed between MDR ESBL-EK and the use of any specific antibiotics.

We performed a secondary analysis in which each subject was included only once (i.e., for the first occurrence of an ESBL-EK). Of the 306 patients included by use of these criteria, 57 (18.6%) had isolates that were MDR ESBL-EK. Multivariable analysis similarly showed that the only independent risk factor for MDR ESBL-EK was infection with *K. pneumoniae* (adjusted OR, 12.23; 95% CI, 4.59–32.59; $P < .001$).

Twenty *K. pneumoniae* isolates and 14 *E. coli* isolates were

Table 3. Multivariable analysis of factors potentially associated with multidrug-resistant extended-spectrum β-lactamase–producing *Escherichia coli* and *Klebsiella* species (ESBL-EK).

Variable	Unadjusted OR	Adjusted OR (95% CI)	P
Total no. of antibiotic-days	…	1.01 (0.99–1.02)[a]	.39
Klebsiella pneumoniae as organism	11.69	11.67 (4.77–28.51)	<.001
Central venous catheter	2.43	1.98 (0.99–3.94)	.05
Duration of hospitalization[b]	…	0.99 (0.98–1.00)[c]	.15

[a] OR reflects the odds associated with each increase in 1 antibiotic-day.

[b] No. of days from hospital admission until infection with ESBL-EK.

[c] OR reflects the odds associated with each increase in 1 day of hospitalization.

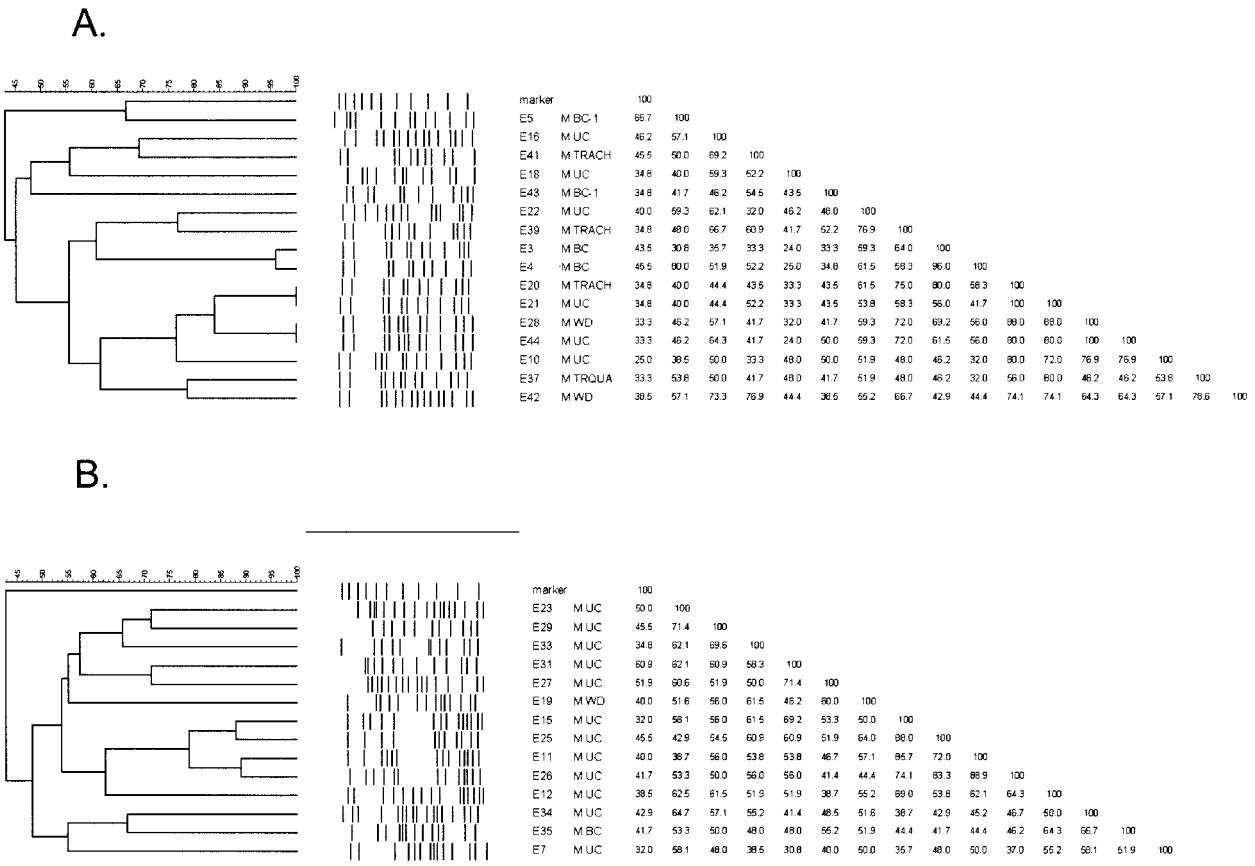

Figure 4. Pulsed-field gel electrophoresis patterns of extended-spectrum β-lactamase-producing *Klebsiella pneumoniae (top)* and *Escherichia coli (bottom)* isolates.

available for molecular analysis. PFGE patterns from the *K. pneumoniae* isolates revealed close genetic relatedness among 8 of the isolates tested. In contrast, there was little evidence of clonality among the 14 *E. coli* isolates tested (figure 4).

DISCUSSION

Of 361 ESBL-EK isolates obtained in the 5.5-year study period, 18.8% were MDR. During the study period, the annual prevalence of MDR among ESBL-EK isolates increased from 12.5% to as high as 26.9%. The only significant independent risk factor for isolation of an MDR ESBL-EK was the species of infecting organism (i.e., *K. pneumoniae*). No association was seen between prior antibiotic use and the MDR ESBL-EK. Molecular analysis indicated close genetic relatedness of *K. pneumoniae* isolates.

As ESBL-EK isolates have increased in prevalence, so have concerns regarding expanded resistance to additional antibiotic classes and thus increased limitation of effective treatments. We calculated a high prevalence of ESBL-EK isolates that also demonstrated resistance to trimethoprim-sulfamethoxazole or fluoroquinolones (77.6% and 57.6%, respectively). Of great concern is the fact that ~1 in 5 ESBL-EK isolates is MDR. Reliance on carbapenems will thus continue to increase because other effective treatment options are increasingly limited. Our results emphasize the importance of identifying risk factors for MDR ESBL-EK to design and implement strategies that will prevent further increases in MDR ESBL-EK.

Infection with *K. pneumoniae* was the only significant independent risk factor for MDR ESBL-EK. There are several possible explanations for this finding. First, *K. pneumoniae* commonly displays higher rates of resistance in general than does *E. coli.* Indeed, data from the institutions participating in this study note that *Klebsiella* species in general display significantly higher rates of resistance to fluoroquinolones and trimethoprim-sulfamethoxazole than do *E. coli* (data not shown). Second, as suggested by our molecular analysis, our results may reflect the spread of ⩾1 MDR-ESBL *K. pneumoniae* clone throughout the hospitals. If this is true, greater emphasis should be placed on early identification of such isolates through active infection-control surveillance. Indeed, in their analysis of bacteremia due to ESBL-producing *K. pneumoniae,* Paterson et al. [10] showed that such infections were less prevalent at institutions that implemented contact isolation for ESBL infections. Our results provide evidence that horizontal trans-

mission may also be a major contributing factor to the emergence of MDR ESBL-EK and suggest that more aggressive infection-control interventions may prevent further dissemination of MDR ESBL-EK.

In addition to the agents included in our definition of MDR, we also noted a high prevalence of resistance to cefepime and piperacillin-tazobactam among our ESBL-EK isolates (93% and 76%, respectively). These resistance rates are somewhat higher than those previously reported among ESBL-producing isolates [30, 31]. Although the use of these agents to treat ESBL-EK infections (when an ESBL-EK is susceptible in vitro) is controversial [32, 33], our data suggest that the opportunities to even consider use of these agents are limited.

Past antibiotic use, as measured in total antibiotic days, was not associated with the development of an MDR-ESBL infection. This finding was unexpected, because ESBL infections have been found to be associated with prior antibiotic use in past studies, including those conducted at our institution [9, 12, 34, 35]. Interventions to limit emergence of ESBL-EK have traditionally focused on restricting certain antimicrobial agents (e.g., third-generation cephalosporins) associated with ESBL infections. Although such interventions are certainly an important component in efforts to control the emergence of ESBL-EK, our results demonstrating clonal strain transmission emphasize that infection-control measures are also likely to be critical in interrupting the spread of such organisms.

Our study had several potential limitations. Although selection bias is of concern in case-control studies, we included all eligible case patients and control patients. Selection bias can also be introduced because of missing charts; however, complete medical records were available for review for 95.6% of the eligible subjects. Selection bias may also have been introduced by allowing subjects to be included >1 time (i.e., if they had ESBL-EK isolates recovered >30 days apart). However, we found no substantive difference in our results when a secondary analysis was conducted in which subjects were included only once. Finally, appropriate debate has surrounded the question of which group represents the optimal control group in case-control studies of antimicrobial resistance (i.e., the general hospital population at risk vs. those patients with the susceptible form of the infection). As suggested by Harris et al. [18], we believe that this depends on the question being asked. Because our primary question was "In patients who develop ESBL infections, what are the risk factors for that ESBL infection demonstrating multidrug resistance?," we selected patients infected with ESBL-EK that did not exhibit MDR as our control patients. A primary concern about selecting as control patients those patients with the susceptible form of the infection is that use of this control group may cause overestimation of the true association between prior antimicrobial use and subsequent resistant infection. In our study, however, we found no such association between antibiotic use and MDR ESBL-EK.

Another potential limitation was that not all ESBL-EK isolates were available for molecular analysis. If the available isolates on which typing was done were different from those isolates that were not available, typing results may not be representative of all ESBL-EK isolates. However, there was no systematic process by which isolates were or were not available. Furthermore, plasmid analysis was not done. Thus, the possibility of dissemination of MDR ESBL-EK through spread of such extrachromosomal genetic elements (even in the presence of unrelated PFGE patterns) cannot be excluded.

Finally, our study was conducted in a large tertiary care medical center and a smaller urban community hospital. Thus, the results may not be generalizable to other institutions.

In conclusion, we determined that 18.8% of ESBL-EK isolates at our institution were MDR. The only independent risk factor for MDR ESBL-EK was infection with *K. pneumoniae*. A large proportion of *K. pneumoniae* isolates were closely genetically related. No association between MDR ESBL-EK and prior antibiotic exposure was found. Our results demonstrate that treatment of ESBL-producing infections will continue to become more complex in coming years because of further limitation of available agents. Enhancement of surveillance for infection control in addition to interventions regarding antimicrobial use is likely to be most successful in curbing the further emergence of MDR ESBL-EK.

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References

1. Knothe H, Shah P, Kremery V, Antal M, Mitsuhashi S. Transferable resistance to cefotaxime, cefoxitin, cefamandole, and cefuroxime in clinical isolates of *Klebsiella pneumoniae* and *Serratia marcescens.* Infection **1983**; 11:315–7.
2. Quinn JP, Miyashiro D, Sahm D, Flamm R, Bush K. Novel plasmid-mediated β-lactamase (TEM-10) conferring selective resistance to ceftazidime and aztreonam in clinical isolates of *Klebsiella pneumoniae.* Antimicrob Agents Chemother **1989**; 33:1451–6.
3. Jacoby GA, Medeiros AA, O'Brien TF, Pinto ME, Jiang H. Broad-spectrum transmissible beta-lactamases. N Engl J Med **1988**; 319:723–4.
4. Meyer KS, Urban C, Eagen JA, Berger BJ, Rahal JJ. Nosocomial outbreak of *Klebsiella* infection resistant to late-generation cephalosporins. Ann Intern Med **1993**; 119:353–8.
5. Rice LB, Willey SH, Papanicolaou GA, et al. Outbreak of ceftazidime resistance caused by extended-spectrum β-lactamases at a Massachusetts chronic-care facility. Antimicrob Agents Chemother **1990**; 34:2200–9.

6. Burwen DR, Banerjee SN, Gaynes RP. Ceftazidime resistance among selected nosocomial gram-negative bacilli in the United States. The National Nosocomial Infections Surveillance System. J Infect Dis **1994**; 170:1622–5.
7. Itokazu GS, Quinn JP, Bell-Dixon C, Kahan FM, Weinstein RA. Antimicrobial resistance rates among aerobic gram-negative bacilli recovered from patients in intensive care units: evaluation of a national postmarketing surveillance program. Clin Infect Dis **1996**; 23:779–84.
8. Nijssen S, Florijn A, Bonten MJM, Schmitz FJ, Verhoef J, Fluit AC. β-Lactam susceptibilities and prevalence of ESBL-producing isolates among more than 5000 European Enterobacteriaceae isolates. Int J Antimicrob Agents **2004**; 24:585–91.
9. Lautenbach E, Patel JB, Bilker WB, Edelstein PH, Fishman NO. Extended-spectrum β-lactamase–producing *Escherichia coli* and *Klebsiella pneumoniae*: risk factors for infection and impact of resistance on outcomes. Clin Infect Dis **2001**; 32:1162–71.
10. Paterson DL, Ko WC, Von Gottberg A, et al. International prospective study of *Klebsiella pneumoniae* bacteremia: implications of extended-spectrum beta-lactamase production in nosocomial Infections. Ann Intern Med **2004**; 140:26–32.
11. Wiener J, Quinn JP, Bradford PA, et al. Multiple antibiotic-resistant *Klebsiella* and *Escherichia coli* in nursing homes. JAMA **1999**; 281:517–23.
12. Lautenbach E, Strom BL, Bilker WB, Patel JB, Edelstein PH, Fishman NO. Epidemiological investigation of fluoroquinolone resistance in infections due to extended-spectrum β-lactamase–producing *Escherichia coli* and *Klebsiella pneumoniae.* Clin Infect Dis **2001**; 33:1288–94.
13. Rahal JJ, Urban C, Horn D, et al. Class restriction of cephalosporin use to control total cephalosporin resistance in nosocomial *Klebsiella.* JAMA **1998**; 280:1233–7.
14. Urban C, Go E, Mariano N, et al. Effect of sulbactam on infections caused by imipenem-resistant *Acinetobacter calcoaceticus* biotype anitratus. J Infect Dis **1993**; 167:448–51.
15. Wong-Beringer A. Therapeutic challenges associated with extended-spectrum, β-lactamase–producing *Escherichia coli* and *Klebsiella pneumoniae.* Pharmacotherapy **2001**; 21:583–92.
16. Bradford PA, Urban C, Mariano N, Projan SJ, Rahal JJ, Bush K. Imipenem resistance in *Klebsiella pneumoniae* is associated with the combination of ACT-1, a plasmid-mediated AmpC β-lactamase, and the loss of an outer membrane protein. Antimicrob Agents Chemother **1997**; 41:563–9.
17. MacKenzie FM, Forbes KJ, Dorai-John T, Amyes SGB, Gould IM. Emergence of a carbapenem-resistant *Klebsiella pneumoniae.* Lancet **1997**; 350:783.
18. Harris AD, Karchmer TB, Carmeli Y, Samore MH. Methodological principles of case-control studies that analyzed risk factors for antibiotic resistance: a systematic review. Clin Infect Dis **2001**; 32:1055–61.
19. Knaus WA, Drapier EA, Wagner DP, Zimmerman JE. APACHE II: a severity of disease classification system. Crit Care Med **1985**; 13:818–29.
20. Garner JS, Jarvis WR, Emori TG, Horau TC, Hughes JM. CDC definitions for nosocomial infections, 1988. Am J Infect Control **1988**; 16: 128–40.
21. NCCLS. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically: approved standard. Wayne, PA: NCCLS, **1997–2002**.
22. NCCLS. Performance standards for antimicrobial disk susceptibility testing: approved standard. Wayne, PA: NCCLS, **1997–2002**.
23. NCCLS. Performance standards for antimicrobial susceptibility testing: approved standard. Wayne, PA: NCCLS, **1997–2002**.
24. Gautom RK. Rapid pulsed-field gel electrophoresis protocol for typing of *Escherichia coli* O157:H7 and other gram-negative organisms in 1 day. J Clin Microbiol **1997**; 35:2977–80.
25. Tenover FC, Arbeit RD, Goering RV, et al. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J Clin Microbiol **1995**; 33:2233–9.
26. Armitage P. Test for linear trend in proportions and frequencies. Biometrics **1955**; 11:375–86.
27. Kleinbaum DG, Kupper LL, Morgenstern H. Epidemiologic research: principles and quantitative methods. New York: Van Nostrand Reinhold, **1982**.
28. Hosmer DO, Lemeshow SL. Applied logistic regression. New York: Wiley & Sons, **1989**.
29. Sun GW, Shook TL, Kay GL. Inappropriate use of bivariable analysis to screen risk factors for use in multivariable analysis. J Clin Epidemiol **1996**; 49:907–16.
30. Yu WL , Chuang YC, Jones RN. A pragmatic approach to identify extended-spectrum β-lactamase–producing *Klebsiella pneumoniae* in Taiwan: in vitro activity of newer and established antimicrobial agents. Diagn Microbiol Infect Dis **2004**; 48:277–82.
31. Jan IS, Hsueh PR, Teng LJ, Ho SW, Luh KT. Antimicrobial susceptibility testing for *Klebsiella pneumoniae* isolates resistant to extended-spectrum β-lactam antibiotics. J Formos Med Assoc **1998**; 97:661–6.
32. Paterson DL. Recommendation for treatment of severe infections caused by Enterobacteriaceae producing extended-spectrum β-lactamases (ESBLs). Clin Microbiol Infect **2000**; 6:460–3.
33. Yu WL, Pfaller MA, Winokur PL, Jones RN. Cefepime MIC as a predictor of the extended-spectrum β-lactamase type in *Klebsiella pneumoniae,* Taiwan. Emerg Infect Dis **2002**; 8:522–4.
34. Pena C, Pujol M, Ricart A, et al. Risk factors for faecal carriage of *Klebsiella pneumoniae* producing extended spectrum β-lactamase (ESBL-KP) in the intensive care unit. J Hosp Infect **1997**; 35:9–16.
35. Kim BN, Woo JH, Kim MN, Ryu J, Kim YS. Clinical implications of extended-spectrum β-lactamase–producing *Klebsiella pneumoniae* bacteraemia. J Hosp Infect **2002**; 52:99–106.