Outbreak of Multiply Resistant Enterobacteriaceae in an Intensive Care Unit: 
Epidemiology and Risk Factors for Acquisition

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A prospective study was initiated in an intensive care unit (ICU) where extended-spectrum \( \beta \)-lactamase–producing enterobacteriaceae (ESBLPE) were endemic. From July 1990 to July 1991, patients hospitalized for \( \geq 5 \) days were screened for ESBLPE acquisition by means of weekly rectal sampling and clinical cultures. Baseline characteristics and various ICU procedures in 62 cases of ESBLPE were compared with those for 205 patients without ESBLPE, with use of Cox’s model. Risk for acquiring ESBLPE (Klebsiella pneumoniae in most cases) increased during the ICU stay, from 4.2% in the first week to 24% in the fourth week. Baseline characteristics were not different between the two groups. Urinary catheterization \( (P = .04) \) and arterial catheterization \( (P = .03) \) were independent risk factors for acquiring ESBLPE and probably reflected frequency of health care manipulations. The first site of ESBLPE acquisition was the digestive tract in 58 of the 62 patients; 28 infections developed in 22 patients, and these followed or occurred simultaneously with rectal colonization in 18 of those 22. DNA macrorestriction analysis suggested that the same strain was responsible for most cases. In conclusion, ESBLPE acquisition depends on length of stay in the ICU and the use of invasive procedures. Colonization is a prerequisite for infection.

Since 1986 several nosocomial outbreaks of extended-spectrum \( \beta \)-lactamase–producing enterobacteriaceae (ESBLPE), mainly Klebsiella pneumoniae, have been reported in France [1–5] and in other countries [6–9]. The resistance is plasmid-mediated and involves all \( \beta \)-lactam antibiotics (except imipenem and cefepime) and most aminoglycosides. In these outbreaks, ESBLPE have been initially isolated from intensive care unit (ICU) patients. Major risks are the extension of ESBLPE out of the ICUs [9] and the spread of resistance to other enterobacteriaceae, trough plasmids, or transposable genes [10]. An outbreak of ESBLPE occurred a few years ago in our ICU [5]. Recently, ESBLPE have been continuously present in ICU patients despite the institution of barrier precautions, leading to a situation of near endemicity. We initiated a prospective cohort study to assess the epidemiology of ESBLPE colonization and infection.

Many studies in the ICU setting have established risk factors for endemic nosocomial infections [11, 12]. Severity of illness at admission and underlying diseases are usually identified as important risk factors. During the ICU stay, nosocomial infections often are associated with invasive procedures at the site of infection, the patient’s severity of illness, and intensity of care. Colonization, however, commonly precedes infection with the same strain. Despite their importance with regard to infection control, risk factors for colonization have infrequently been studied and may differ from those for infection. The second purpose of this study was to identify risk factors for acquiring ESBLPE in an ICU.

Methods

Patients

Bichat-Claude Bernard University Hospital (Paris) is a 1,200-bed tertiary-care teaching hospital and handles \( \sim 30,000 \) admissions per year. The Infectious Diseases Department includes an 18-bed ICU that admits 400–450 patients per year. Approximately one-half of the patients are referred from other hospitals, 30%–35% from other units of the hospital, and 10%–15% from the emergency department. Most patients are admitted because of a community-acquired infection or a nosocomial infection following major elective surgery or another ICU stay. Approximately 50% of patients are immunocompromised because of HIV infection or chemotherapy.

All patients admitted from 1 July 1990 through 31 July 1991 who remained hospitalized for \( \geq 5 \) days in the ICU were included in our study. This minimum length of stay was based on the assumption that the risk for acquiring ESBLPE was low during the first days of stay [13] and that these patients might have very few, if any, surveillance specimens obtained during their stay. In addition, neutropenic patients who were routinely receiving oral antimicrobial prophylaxis and patients colonized with ESBLPE at admission were excluded. Only the first ICU admission for each patient was included in our analysis.
All ESBLPE colonizations or infections diagnosed during the ICU stay or up to 2 days after discharge from the ICU were included. For each patient, the following data were collected: age, gender, length of hospital stay before transfer to the ICU, primary diagnosis, simplified acute physiology score within 24 hours of admission [14], severity of underlying disease as described by McCabe and Jackson [15], organ system failure after 48 hours in the ICU [16], and immunodepression. During the ICU stay, data regarding the use and duration of the following devices and procedures (if they lasted for >2 days) were collected: mechanical ventilation; central venous, pulmonary artery (Swan-Ganz), or arterial catheterization; urinary catheterization; nasogastric tube placement; enteral feeding; and administration of systemic antimicrobial therapy.

Microbiological Surveillance

A rectal swab specimen and urine sample were obtained once and twice a week, respectively, throughout the patient’s ICU stay. Surveillance specimens were not obtained upon admission to the ICU. Samples were cultured on a selective medium for gram-negative bacilli (Drigalski lactose agar; Sanofi Diagnostics Pasteur, Marne la Coquette, France), supplemented with cefotaxime (0.5 mg/L). Urine samples were quantitatively cultured; the minimal detection level of ESBLPE in fecal samples was 10^2 cfu per gram of stool. Other specimens were taken for clinical reasons when considered necessary by the medical staff. Samples were routinely cultured, and standardized methods for detection of enterobacteriaceae (API System; bioMérieux, Marcy l’Étoile, France) were used. ESBLPE from surveillance cultures and other specimens were identified with use of the disk-diffusion method. They were detected by resistance (or decreased susceptibility) to third-generation cephalosporins and most aminoglycosides, as well as by the synergy between disks containing cefotaxime, ceftazidime, and aztreonam and a disk containing amoxicillin/clavulanic acid [1, 4, 17]. To determine their molecular relatedness, ESBLPE isolates from patients whose digestive tract was colonized and whose specimens from other site(s) were culture-positive were subjected to DNA restriction analysis with pulsed-field gel electrophoresis. Extraction, purification, and restriction of chromosomal DNA with Xba I were performed as recently described [18].

Definitions of Colonization and Infection

Pulmonary infection was defined by the presence of a new and persistent infiltrate as evidenced on chest roentgenography; purulent tracheal aspirate; and at least 10^5 cfu/mL in culture of a protected-brush specimen [19]. A urinary tract infection was defined by a positive culture yielding ≥2 types of organisms (≥10^5 cfu/mL). A diagnosis of catheter-related infection was considered if (1) semiquantitative culture of the catheter tip yielded at least 10^5 cfu/mL [20] or (2) general signs of infection were associated with a positive culture of the catheter tip (whatever the bacterial count) and there was no other obvious cause of infection. Primary septicemia was defined by the positivity of ≥1 blood culture without an obvious primary focus of infection. Secondary septicemia was recorded with the primary focus of infection. Wound or cutaneous infection was considered when a positive culture was associated with erythema and/or purulence at a site.

If these criteria for infection were not met, the presence of ESBLPE was considered as colonization. A rectal swab culture yielding ESBLPE was considered evidence of colonization. A maximum of one infection or colonization was recorded per site. If subsequent infection developed after colonization at the same site, only the infection was recorded.

The following barrier precautions for personnel caring for ESBLPE-infected or -colonized patients were instituted: identification of carriers, use of gloves and aseptic handwashing during/after contact with colonized patients or their environment, and use of gowns for close contact with colonized patients.

Cultures of Environmental and Hand Specimens

During one cluster of ESBLPE acquisitions, environmental screening was performed in rooms of ESBLPE-infected patients. Water-moistened swabs were used to sample horizontal surfaces and knobs of EKG monitors, mechanical ventilators, continuous-infusion pumps, other points of frequent hand contact (door handles, taps, trolleys, blood pressure cuffs), and bedrails. During the same period, specimens from the hands of nursing personnel and medical staff were cultured immediately after their contact with ESBLPE-positive patients (after glove removal and before handwashing). Swabs and fingertips were applied on the same selective medium agar used for surveillance specimens.

Statistical Analysis

The time to occurrence of ESBLPE acquisition (i.e., first isolation of organisms identified as ESBLPE) was calculated from the date of ICU admission, on the basis of the Kaplan-Meier estimate [21] and actuarial life table methods [22]. Patients without ESBLPE were censored at the time of their discharge or death. Through the log-rank test [23], we first studied the predictive value (for ESBLPE acquisition) of several baseline variables, i.e., those assessed within the first 48 hours of the ICU stay: demographic characteristics, reason for ICU admission, and severity of illness. A Cox’s model was then applied to summarize prognostic information and estimate relative risk (RR), reported herein with 95% confidence intervals (95% CIs) [24]. Second, the prognostic value of the use of several intensive procedures during the ICU stay was studied with use of Cox’s model, in which each procedure was intro-
Figure 1. Estimate of the ratio of patients acquiring extended-spectrum β-lactamase-producing enterobacteriaceae (ESBLPE) over the study period. Ratios over bars denote the number of new monthly cases over the number of exposed patients (staying for at least 5 days in the intensive care unit) who were admitted during that month.

Results

During the study period, 474 patients were admitted to the ICU. Of those, 188 (40%) were discharged within the first 4 days of the ICU stay. Of the 286 eligible patients, 19 were excluded because of colonization at the time of admission (4 patients) or incomplete information (15 patients, 2 with and 13 without ESBLPE). Thus, 267 patients were included in the study cohort. One hundred and ninety-five patients (73%) were referred to the ICU for an infectious condition, and 97 (36%) were infected with HIV. The main type of dysfunction was respiratory in 115 patients (43%), neurological in 67 (25%), cardiac or hemodynamic in 56 (21%), and of another nature in 29 (11%). Sixty-nine patients (26%) died during the ICU stay. The median ICU stay was 13 days (range, 5–147 days).

During the study period, 62 patients acquired ESBLPE, a crude incidence estimated at 23% (95% CI, 18%–28%). In sixty (97%) of the 62 patients with ESBLPE, the multiresistant strain was K. pneumoniae. The first site of isolation was the digestive tract (n = 58), the urinary tract (n = 3), or a central venous catheter (n = 1). The monthly incidence of acquisition varied over the 13-month study period, with the baseline occurrence of an average of 2 cases per month and 2 clusters (in October 1990 and March 1991) (figure 1). The Kaplan-Meier estimates of time to colonization are shown in figure 2A, as well as the conditional probabilities that a patient would be colonized at a particular time (given that he or she had not been colonized previously) (figure 2B). It is shown that the latter roughly increased over time, with an 8% conditional probability of acquisition from day 12 to day 17 and an estimated 30% probability from day 44 to day 60.

At admission none of the following factors were associated with more-rapid or more-frequent ESBLPE acquisition (table 1): age, gender, duration of hospital stay, infectious disease or HIV diagnosis, and severity of illness. At admission to the ICU, 101 patients were mechanically ventilated and 38 required placement of an arterial catheter; 50, of a central venous catheter; 23, of a pulmonary artery catheter; and 131, of nasogastric tubes. After admission, additional patients required ventilatory assistance (n = 51); the use of arterial (n = 49), urinary (n = 173), central venous (n = 69), or pulmonary artery (n = 52) catheters; or the use of nasogastric tubes (n = 40). Broad-spectrum β-lactam antimicrobials were administered to 104 patients (54 received them at admission).

The use of arterial (RR = 3.0; 95% CI, 1.4–6.4; P = .01), urinary (RR = 1.9; 95% CI, 1.1–3.3; P = .01), central venous (RR = 1.7; 95% CI, 1.0–2.9; P = .04), or pulmonary artery catheters (RR = 2.2; 95% CI, 1.0–4.8; P = .06) or the administration of broad-spectrum β-lactam antimicrobials (RR = 1.7; 95% CI, 1.0–2.8; P = .05) was individually associated with the outcome. By contrast, the use of nasogastric tubes (RR = 1.6; 95% CI, 0.9–2.9; P = .09), enteral feedings (RR = 0.9; 95% CI, 0.3–1.6; P = .8), or mechanical ventilation (RR = 1.3; 95% CI, 0.8–2.1; P = .4) was not associated with ESBLPE acquisition. When these factors were assessed in multivariate analysis, only the use of arterial (P = .03) or urinary catheters (P = .04) remained significantly associated with ESBLPE acquisition.

ESBLPE was isolated from a site other than the digestive tract in 22 patients (32 positive cultures). Four of the 32 positive cultures yielding ESBLPE indicated colonizations (wound, 3; trachea, 1) and 28 indicated infections; these included urinary tract (n = 14), pulmonary (n = 6), catheter-related (n = 4), primary bloodstream (n = 2), and cutaneous or wound infections (n = 2). The digestive tracts of 18 of these 22 patients were previously (n = 16) or simultaneously (n = 2) colonized. The median interval from digestive tract colonization to infection was 5 days (range, 0–24 days). Four patients acquired infection without previous digestive tract colonization: three of the patients remained free of digestive tract colonization, whereas a rectal swab specimen from one patient was positive 4 days after the first infection. In March 1991, 18 environmental swab specimens were obtained from three rooms of ESBLPE-infected patients; all were negative for ESBLPE, as were 26 hand-specimen cultures.

ESBLPE isolates from 15 patients with digestive tract colonization and subsequent infection(s) were available for DNA macrorestriction analysis. Three different restriction endonuclease analysis profiles were identified (figure 3). One pattern...
Figure 2. Estimation of (A) time to and (B) instantaneous risk of acquisition of extended-spectrum β-lactamase–producing enterobacteriaceae (from time of admission to the intensive care unit). In parentheses are the number of exposed patients on days 10, 20, 30, 40, and 50 after admission.

(genotype profiles B₁, B₂, and B₃) was shared by 13 patients, and two different patterns (profiles A and C) were identified in one patient each. For 13 patients the profile of the infecting strain(s) was identical or very similar to that of the colonizing strain, differing by one or two bands for two patients (profiles B₁ to B₂ and B₁ to B₃, table 2 and figure 3).

Discussion

Outbreaks of nosocomial infection due to ESBLPE were initially reported by investigators in Europe, occurring especially in the ICU setting [1–5]. Recently, outbreaks have been reported in North America [25], occurring in ICUs but also in general wards [6, 7] and nursing homes [8, 9]. Data from the National Nosocomial Infections Surveillance System showed that in 1993, 14.2% of K. pneumonia isolates were likely to harbor an extended-spectrum β-lactamase [26]. The increasing number of these outbreaks underlined the need for a better understanding of ESBLPE acquisition and infection.

The first goal of this study was to define the epidemiology of ESBLPE in ICU patients. Knowledge of the epidemiology of nosocomial infections in ICUs is based essentially on reports of outbreaks [27]. ESBLPE had been present in our ICU for several years, initially with transient clusters. Recently, ESBLPE have been continuously isolated despite standard barrier precautions. This near-endemicity provided the opportunity to study colonization over a prolonged period.

ESBLPE were isolated from 23% of ICU patients staying for at least 5 days. This high frequency was detected only because of systematic gastrointestinal tract surveillance. Without such surveillance, the number of patients acquiring ESBLPE would have been greatly underestimated; only 22 of 62 colonized patients had ESBLPE-positive clinical cultures. The rectum was the main site of colonization, a finding in agreement with previous studies showing that endogenous flora constitute the major reservoir of multidrug-resistant enterobacteriaceae in ICU patients [28–30].

Unfortunately, only strains from 15 of the 62 carriers were available for DNA analysis. The patients from whom they were isolated, however, were hospitalized at different times.
throughout the study period. The results of the DNA analysis showed that one strain of ESBLPE was responsible for 13 of the 15 cases. There was at least one colonized patient in the unit at any time during the study period. In addition, the environment was sampled for culturing during one of the two clusters: all of these cultures (albeit there were few) were negative for ESBLPE. These results suggest that cross-transmission by hand carriage from patient to patient was the major mode of ESBLPE acquisition [28, 31].

Infection due to ESBLPE developed in 22 patients and followed digestive tract colonization in 18. Urinary tract infections accounted for one-half of the infections and developed in two-thirds of the infected patients, suggesting that the rectum—rather than the upper digestive tract—was the main site of colonization with ESBLPE in this ICU population. ESBLPE from 15 of these 18 patients were available for DNA analysis. All patients' infecting and colonizing strains had the same or a closely related DNA banding pattern. For two patients there was a difference of one or two bands between the colonizing and infecting strains. These isolates could be considered identical, since chromosomal evolution was likely to account for these minor variations. These data reinforce those from previous studies using phenotypic methods that demonstrated that colonization with antibiotic-resistant enterobacteriaceae in most instances precedes infection with the same strain [28, 29, 32].

The other goal of the study was to identify risk factors for ESBLPE acquisition. None of the parameters assessed at ICU admission were associated with ESBLPE acquisition. Other studies generally found severity of underlying disease or gravity scores at admission as risk factors for nosocomial infections [11, 12]. Risk factors for colonization could differ from those for infection. Patients with severe underlying diseases may

Table 2. Molecular characterization of extended-spectrum β-lactamase-producing \( K. \) pneumoniae: Distribution according to macrorestriction genotypes.

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Colonization</th>
<th>Infection</th>
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<tbody>
<tr>
<td>1–11</td>
<td>( B_1 )</td>
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<td>12</td>
<td>( B_1 )</td>
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<td>15</td>
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Figure 3. Profiles produced by pulsed-field gel electrophoresis of \( Xba \) I macrorestriction fragment of extended-spectrum β-lactamase-producing \( K. \) pneumoniae isolated from 15 patients. Lanes 1 and 14: molecular size marker (lambda DNA concatemer, Bio-Rad). Lanes 2 and 3: genotype A (patient 14). Lanes 4, 5, 8, and 9: genotype \( B_1 \) (patients 1–11). Lanes 6 and 7: genotypes \( B_1 \) and \( B_2 \) (patient 12). Lanes 10 and 11: genotypes \( B_1 \) and \( B_3 \) (patient 13). Lanes 12 and 13: genotype C (patient 15).
have altered host defenses and are therefore at risk for nosocomial infection. Conversely, host defenses are of little, if any, importance in colonization by a natural inhabitant of the digestive tract.

The rate of ESBLPE acquisition increased with ICU length of stay, and nearly one-half of the patients were ESBLPE-positive after 30 days in the ICU. The length of stay in the ICU was probably a major risk factor for ESBLPE acquisition. Use of arterial and urinary catheters was independently associated with the acquisition of ESBLPE during the ICU stay. It is interesting that a large study found the same risk factors for all nosocomial infections in the ICU [11]. There was no clear relationship between these two invasive procedures and the digestive acquisition of ESBLPE. We postulate that they have to be considered as markers for severity of illness and nursing manipulations. Although ESBLPE were not found on the hands of personnel, this route of transmission has been extensively documented [28]. Therefore, the risk of ESBLPE acquisition by cross-transmission probably increases with health-care-worker contact. An analysis including a nursing-manipulations scoring system [33] could help determine the role of health-care-worker contact in the acquisition of multidrug-resistant enterobacteriaceae.

In our study, treatment with broad-spectrum β-lactam antimicrobials was not a risk factor for ESBLPE acquisition. Previous studies identified antimicrobial exposure as a risk factor for infections with antimicrobial-resistant enterobacteriaceae [12, 34, 35]. In particular, ceftazidime therapy has been implicated in several ESBLPE outbreaks; outbreaks were associated with the increasing consumption of this antibiotic and were terminated when ceftazidime was restricted [6, 7, 9]. These studies, however, did not control for other risk factors and interventions [36]. Starting in January 1992, infection control interventions in our ICU included reinforcement of barrier precautions and transient use of selective digestive decontamination [13], whereas antimicrobial use was not restricted. These interventions will be described in detail elsewhere. With use of the same surveillance methods (weekly rectal specimen and urine screening), incidence of ESBLPE acquisition significantly decreased over 3 years (figure 4). The fact that the outbreak was progressively controlled without antibiotic restriction adds to the evidence that barrier precautions are the most important measures to be implemented in such situations.

In conclusion, this study confirms the epidemiology of antimicrobial-resistant enterobacteriaceae in ICU patients. One ESBLPE strain was responsible for most of the ICU-acquired cases. Colonization was a prerequisite for infection. Risk factors for ESBLPE colonization are probably related to the duration of exposure to an endemic nosocomial strain and health care manipulations. None of the risk factors were modifiable. Our results emphasize the necessity of early detection of ESBLPE colonization and reinforcement of barrier precautions in preventing ESBLPE cross-transmission.

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References

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