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Prospective survey of colonization and infection caused by SHV-4 producing *Klebsiella pneumoniae* in a neurosurgical intensive care unit

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SUMMARY

The occurrence of extended-spectrum β-lactamase producing enterobacteria (ESBLE) has been prospectively surveyed in a neurosurgical intensive care unit (ICU). Of the 47 patients examined, 8 were identified as faecal carriers, and 2 of them developed a subsequent urinary tract infection. ESBLE were also detected in the immediate environment of five colonized and/or infected patients. All isolates were *Klebsiella pneumoniae* of a particular biotype which exhibited a similar antibiotype and produced an SHV-4 type β-lactamase. However, plasmid profiling and ribotyping revealed that strains isolated from seven patients of hall A were a single epidemic clone, whereas strains isolated from the eighth patient of hall B were different. Comparison between the characteristics of patients who carried an ESBLE during the surveillance period, and control patients who did not, showed that a recent surgery, and the length of ICU stay were significantly associated with the acquisition of ESBLE.

INTRODUCTION

Since 1984, nosocomial outbreaks of extended-spectrum β-lactamase producing enterobacteria (ESBLE) have been increasingly reported in France [1–4] and in other countries [5, 6]. The majority of these organisms were isolates of *Klebsiella pneumoniae*, often collected from patients hospitalized in intensive care units (ICU). Among extended-spectrum β-lactamases (ESBL) which are essentially TEM or SHV type β-lactamases [7, 8], some have been described worldwide, such as SHV-2 and SHV-5, whereas others have been reported only in one or few countries, for example TEM-10 and TEM-12 in the United States [9] and TEM-3, SHV-3 and SHV-4 in France [2, 10–14].

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Infection control efforts aim at identifying the source and the mode of transmission of epidemic nosocomial strains. However, outbreaks due to ESBLE are usually complex phenomena, which may involve simultaneously strain and plasmid or even gene dissemination [15–17]. Consequently, their epidemiologic investigation requires suitable methods: after the β-lactamase characterization, the use of plasmid analysis together with a chromosomal marker, is essential. Molecular techniques applied to chromosomal analysis of *K. pneumoniae* have included ribotyping [14, 15], analysis by pulsed-field gel electrophoresis [14, 16, 18], or random amplified-polymerase chain reaction [19].

Since 1987, nosocomial infections caused by ESBLE have been observed with an increasing frequency in

Pellegrin Hospital (Bordeaux, France). In 1993, 27% of the ESBLE collected in this hospital came from the same neurosurgical ICU [20], despite the motivation of the healthcare staff. In order to elucidate the emergence and/or dissemination of ESBLE in this unit, a 3-month prospective survey was initiated at the end of 1994. The ESBLE were systematically screened in clinical and environmental samples, and the isolated strains were studied by phenotypic and genotypic markers to establish their epidemiological relation. The characteristics of the patients were analysed in order to identify the risk factors for acquiring ESBLE in this ICU.

MATERIALS AND METHODS

Setting

Pellegrin Hospital is a 1518-bed university-affiliated hospital that mainly includes surgical units (950 beds). The neurosurgical ICU consists of a 12-bed unit divided into 2 areas (halls A and B), each of 6 beds and 4 individual cubicles. There are a lot of contacts between patients of halls A and B. Indeed, hall A accommodates patients requiring strict intensive care, generally coming from the operating theatre suite; hall B is intended for chronically-ill patients, mainly coming from hall A when less intensive care is required. The average number of admissions to ICU per year is 500 patients, of all ages but mainly adults. These patients are admitted for pre- and post-neurosurgical intensive care, often because of complications following surgery for cerebral tumour or vascular disease. The mean length stay in ICU is 12 days. The great majority of patients who stay more than 48 h are on mechanical ventilation and enteral feeding.

Surveillance

All adult patients ($\geqslant$ 18 years) admitted from 15 October 1994 to 15 January 1995 who remained hospitalized for more than 48 h in this ICU, were included in the study. This minimum length of stay was based on the assumption that the risk for acquiring ESBLE is low during the first days of stay [21]. All patients were sampled immediately on admission to the ICU, but specimens were stored at 4 °C and only processed if the patients stayed more that 48 h. Then, clinical specimens were collected systematically, once a week for stools and urines, and twice weekly for tracheal secretions. In addition, other specimens (i.e. blood cultures) were recovered when signs of infection (as defined by the CDC [22]) were evident. A stool culture yielding an ESBLE was taken as an evidence of colonization.

Samples from the immediate environment (rooms) of patients colonized and/or infected with ESBLE were obtained once a week. Several points were tested: floor, sheets, urine collection bags, door handles, bed railings, staff gowns, and various items of equipment, including ventilator tubing. The distant environment (ward) of all patients was also sampled four times during the study.

For each patient, the following data were collected: age, sex, previous hospital stay, reason for ICU admission, severity scores on admission, intensity of cares, invasive procedures, and administration of systemic antimicrobial therapy. Controls were patients of ICU who did not carry an ESBLE during the surveillance period. The comparison of the different parameters was done by χ^2 test. P values of 0·05 or less were considered as significant.

Strain selection

All samples were examined for the presence of ESBLE by plating on selective medium, i.e. Drigalski agar (Diagnostics Pasteur, France) supplemented with 2 mg/l of ceftazidime [23]. ESBLE were identified using the API 20E system (BioMérieux SA, France). All isolates were antibiotyped, and 18 representative strains (the first isolate from each patient, each sample source and each antibiotic resistance pattern) were selected and analysed further for molecular epidemiological study. Three *K. pneumoniae* strains isolated from another hospital were used as unrelated control strains for ribotyping experiments.

Antibiotic resistance testing

The production of an ESBL was detected by the synergistic effect of the combination of clavulanic acid-amoxicillin with cefotaxime, ceftazidime, cefpirome, and/or aztreonam in the double-diffusion test [24]. Antibiotic resistance tests were performed by the disk diffusion method on Mueller–Hinton agar, according to the recommendations of the French Society of Microbiology [25]. Forty-two antibiotic disks (Diagnostics Pasteur, France) were used, including 15 β-lactam agents and 12 aminoglycosides.

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Analytical isoelectric focusing

β-Lactamases were released by ultrasonication treatment and their isoelectric points (pI) were determined by isoelectric focusing in polyacrylamide gels, using reference enzymes of known pI as markers [26]. The β-lactamase activities were detected by the iodine procedure in gel using successively benzylpenicillin (0·125 g/l) and ceftriaxone (0·25 g/l).

Plasmid and chromosomal analysis

Plasmid DNA and total DNA was extracted as previously described [4]. The *Eco*RI (Bethesda Research Laboratories, France) restriction, agarose gel electrophoresis, Southern transfer, and DNA–DNA hybridization with ^{32}P-labelled probes under stringent conditions were performed as reported elsewhere [4]. DNA probes specific of β-lactamase genes were obtained by gel purification of a 560 bp *Pst*I/*Ssp*I fragment of pBR322 plasmid for $bla_{\text{TEM-1}}$ [27], and of a 453 bp *Pst*I/*Not*I fragment of pHUC37 plasmid for $bla_{\text{SHV-3}}$ [28]. The probe used for ribotyping was the pKK3535 plasmid, a pBR322 derivate plasmid containing the entire *rrn*B operon [29]. Ribotyping experiments were done at least twice to ensure the reproducibility of the method.

RESULTS

Epidemiological data

During the 3-month study period, 99 adult patients were hospitalized in the neurosurgical ICU. Of them, 52 were discharged within the first 48 h of hospitalization. Thus, 47 patients were included in this study (25 females and 22 males), with a mean age of 56·8 years. Their median ICU stay was 48 days. The main cause of their hospitalization was vascular pathology (69%). A total of 393 clinical (163 stools, 150 urines and 80 tracheal secretions) and 355 environmental samples was recovered, including 175 samples from the immediate environment of the colonized and/or infected patients.

Description of the colonized and/or infected patients

Fifty-four clinical samples (51 stools and 3 urines) positive for an ESBLE were obtained from 8 patients (named A–H), 5 females and 3 males with a mean age of 57 years (range, 38–78 years). All carried ESBLE in their stools; 2 of them (patients C, H) developed a

Table 1. *Phenotypic and genotypic characteristics of ESBL producing* K. pneumoniae

Patient	No. and source of isolates			ESBL typing						Ribotype
	Stool	Urine	Env	ATB	Resistance markers	pI	Probe		Plasmid profile	
							SHV	TEM		
A	3	—	2*†	A1	SmSpKNmTANt	7·8	+	—	P1	E1
B	6	—	1‡	A1	SmSpKNmTANt	7·8	+	—	P1	E1
C	11	1	1‡	A1	SmSpKNmTANt	7·8	+	—	P1	E1
D	1	—	—	A1	SmSpKNmTANt	7·8	+	—	P1	E1
E	9	—	1*	A1	SmSpKNmTANt	7·8	+	—	P1	E1
F	2	—	—	A1	SmSpKNmTANt	7·8	+	—	P1	E1
G	1	—	—	A1′	SmSpKNmTANt	7·8	+	—	P1	E1
	3	—	—	A1″	SmSpKNmTANt	7·8	+	—	P1	E1
H	5	—	1‡	A2	KTANt	7·8	+	—	P2	E2
	6	—	—	A2′	KTANt	7·8	+	—	P2	E2
	4	2	—	A2″	KTANt	7·8	+	—	P2	E2

Key: ATB, antibiotype; Sm, streptomycin; Sp, spectinomycin; K, kanamycin; Nm, neomycin; T, tobramycin; A, amikacin; Nt, netilmicin; Tc, tetracycline; Cm, chloramphenicol; Su, sulphonamides; Tp, trimethroprim; Na, nalidixic acid; Ofx, ofloxacin; Fos, fosfomycin; Mox, moxalactam resistance; Env, environmental location; SHV, $bla_{\text{SHV-3}}$; TEM, $bla_{\text{TEM-1}}$.
* Soil. † Bed railing. ‡ Ventilator tubing.

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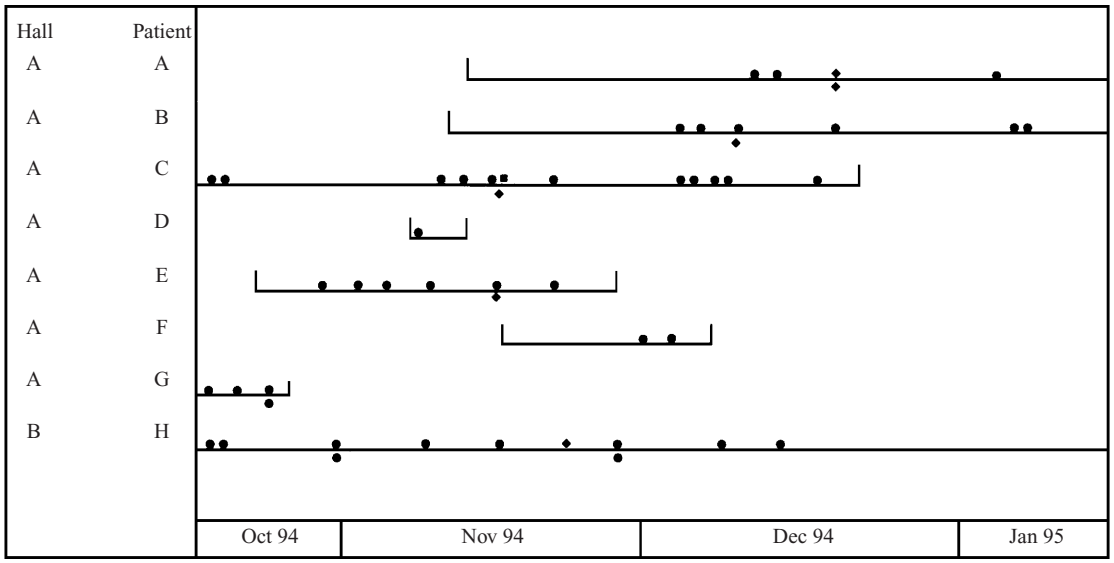

Fig. 1. Distribution over time in the neurosurgical ICU of the eight patients colonized and/or infected by ESBL producing *K. pneumoniae*. ⌊——⌋, Hospitalization period of patient; ●■◆, isolation of ESBLE in stool, urine, and environment, respectively.

subsequent urinary tract infection, associated with indwelling urinary catheters. No isolate was collected from the respiratory tract (even though 7 were receiving mechanical ventilation), or from any other site. The environmental analysis revealed 6 strains which were isolated in the immediate environment of 5 patients (A, B, C, E, H): 3 from ventilator tubing, 2 from the floor, and 1 from a bed railing (Table 1). The time distribution of the ICU stay of all 8 patients, in particular of the 7 patients hospitalized in hall A (patients A–G), showed an overlap (Fig. 1). Three patients (patients C, G, H) harboured the ESBLE at the beginning of the study. The length of their ICU stay, was 20, 76 and 433 days. One patient (D) harboured the ESBLE on readmission into the unit. This patient had been hospitalized in the ICU 2 months previously, and stayed at home between the two hospitalizations. The four remaining patients (A, B, E, F) acquired the ESBLE after a mean ICU stay of 20 days (range, 10–24 days). All patients came from the community, except for G who was transferred from a smaller hospital. Thus, during this study, the rate of ESBLE acquisition was 8·5% (4 of 47 patients). Clinical data obtained for the 7 patients who harboured an ESBLE during the study period (excluding patient D, who was positive on re-admission and stayed only 72 h), and for 22 control patients who did not, were compared (Table 2). Two parameters appeared to be significantly related to ESBLE acquisition: (i) a recent surgical intervention (100% for patients with ESBLE and 50% for control patients) and, (ii) the length of ICU stay, much longer for EBSLE patients (80 days) than for control patients (32 days).

Antibiotic resistance phenotype analysis

All the 60 clinical and environmental ESBLE belonged to the *K. pneumoniae* species, with a particular biotype (weakly urease reaction and sucrose negative). They were highly resistant to all β-lactam agents except for cephamycins and carbapenems, and exhibited a ceftazidime (CAZ) phenotype [26]. Three of these strains, isolated at the end of the study, showed a decreased suceptibility to moxalactam. All isolates were resistant to kanamycin, tobramycin, amikacin and netilmicin, which is consistent with the production of a 6′-aminoglycoside acetyltransferase type IV [AAC(6′)-IV]. Based on resistances to other aminoglycosides, two main antibiotypes were defined (Table 1): ATB A1, including 44 isolates from patients A–G, and ATB A2, including 16 isolates from patient H. Minor variations were observed within ATB A1 for isolates of patient G (sub-antibiotypes ATB A1′ and A1″) and within ATB A2 for isolates of patient H (sub-antibiotypes ATB A2′ and A2″).

ESBL analysis

By electrofocusing analysis, the 18 clinical and environmental representative isolates harboured two bands of β-lactamase activity: one band of pI 7·6

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Table 2. *Comparison of risk factors of patients with or without ESBLE*

Variable	No. (%) of patients	
	With ESBLE ($n = 7$)	Without ESBLE ($n = 22$)
Age (yr)	56·7 ± 15·8	38·9 ± 23·2
Sex (female/male)	4/3	11/11
Previous hospital stay	1 (14)	4 (18)
Reason for ICU admission		
Vascular disease	4 (50)	15 (68)
Tumour	2 (25)	4 (18)
Mean ICU stay (days)	80*	32*
Surgical intervention	7 (100)*	11 (50)*
Antibiotic therapy (AMC/3GC)	4 (57)	11 (50)
Severity scores on admission		
Glascow†	8	6·6
IGS†	7·7	10·3
Intensity of cares		
Ω score†	258	269
Invasive procedures		
Intubation	6 (86)	19 (86)
Urinary catheter	6 (86)	15 (71)
Venous central catheter	4 (57)	7 (33)

Key: AMC, amoxicillin + clavulanate acid; 3GC, third generation cephalosporin.
* $P < 0{\cdot}05$ when compared with the non-colonized group.
† Glascow and IGS are simplified acute physiology scores [32] and Ω score is a therapeutic index proposed to evaluate the nursing workload [32].

corresponding to the chromosomal β-lactamase of *K. pneumoniae* [14, 15], and another band of pI 7·8. The latter enzyme, expected to be an ESBL since it was also detected when ceftriaxone was the substrate, co-migrated with the SHV-4 enzyme used as reference (data not shown). Plasmid DNA hybridized with the SHV probe but not with the TEM probe.

Genotypic analysis

The plasmid analysis of all representative ESBLE showed that they contained a single large plasmid (data not shown). After *Eco*RI restriction, two patterns P1 and P2 were obtained. The profile P1 (~ 180 kb, ⩾ 25 bands, range 12·5–1·4 kb) was given by isolates of the seven patients A–G. The profile P2 (~ 85 kb, ⩾12 bands, range 15·0–2·1 kb) was very distinct from P1, and was observed for isolates of the remaining patient H (Table 1). Hybridization with the SHV probe mapped the gene on two *Eco*R1 DNA fragments, of about 12 and 8 kb for plasmids of the P1 type, and of about 15 and 11 kb for plasmids of the P2 type.

The ribotyping analysis, after restriction with *Eco*RI revealed that the strains divided into two patterns: ribotype E1 (12 bands, range ~ 11·5–0·9 kb) obtained from isolates of patients A–G, and ribotype E2 (14 bands, range ~ 11·5–0·9 kb) from isolates of patient H. These two profiles showed only 6 common bands (Table 1), and were also very different from the 3 other ribotypes given by the 3 epidemiologically unrelated *K. pneumoniae* strains used as controls (data not shown). These results were highly reproducible throughout several assays.

DISCUSSION

In 1993, 27% of ESBLE collected in our hospital came from a neurosurgical ICU [20]. In order to examine the occurrence of ESBLE in this unit, a prospective survey was undertaken during a 3-month period at the end of 1994. Among the 47 patients admitted in this unit during more than 48 h, 8 were identified as faecal carriers. This was a very high colonization rate, since it corresponded to the presence of 3 (25%)–4 (33%) carriers in this 12-bed unit, all through the surveillance period. However, only two patients became infected, developing a urinary tract infection. Colonization is known to precede infection

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[21]. Our observation re-emphasizes the need for identifying the pool of faecal carriers of ESBLE in a ward, as soon as a single patient become infected by an ESBLE, in order to determine the extent of dissemination of such strains.

The first goal of this study was to determine whether this endemic situation was due to one or several strain(s), and to elucidate their reservoir and mode of transmission as a step toward infection control. All 60 ESBLE collected from the 8 patients were strains of *K. pneumoniae*, of a particular biotype, exhibiting a CAZ phenotype and resistant to most aminoglycosides including amikacin. However, with an extended antibiogram, these strains divided into two main antibiotypes, ATB A1 and ATB A2; minor variations, observed within each group, might be related to chromosomal mutations altering the target and/or the permeation of antibiotic(s). Thus, moxalactam resistant strains of patient G were isolated after moxalactam treatment. Antibiotic resistance pattern is an epidemiological marker easy to perform, but highly unstable under selection pressure; discrimination depends on the number of antibiotics tested. The 18 representative strains expressed an ESBL most probably of the SHV-4 type, since this β-lactamase is the single SHV-derived enzyme with a pI of 7·8, described at present [7]. Moreover, SHV-4 is commonly encountered in France [14] and was produced by 96% of the ESBLE found in our hospital in 1993 [20]. Currently the epidemiological investigation of nosocomial outbreaks requires, for certainty, the use of genotypic markers. Plasmid analysis and a chromosomal marker such as ribotyping are necessary to differentiate plasmid and/or strain outbreaks [15–17]. Both markers, determined for the 18 representative strains, gave concordant results and demonstrated the existence of a clonal strain with the plasmid profile P1 and ribotype E1 in the seven patients of hall A, whereas the strains isolated from the eighth patient of hall B were quite different (plasmid profile P2, ribotype E2). Plasmid fingerprinting has been recognized as a good method to analyse outbreaks of short duration [15], and ribotyping reveals stable genetic differences with a high degree of reproducibility, particularly for *K. pneumoniae*, as confirmed here. Consequently, we concluded that there was a single ESBLE strain disseminated in hall A, but patient H, hospitalized since more than 2 years before in hall B, harboured another strain of ESBLE, and was not the source of the outbreak, as initially thought.

The reservoir of the epidemic strain (ATB1, P1, E1) appeared to be the digestive tract of colonized and/or infected patients, as previously observed for nosocomial multiresistant *Enterobacteriaceae* [30]. Our study confirms that the bowel is the elective site for the ESBLE within the digestive tract, since in patients on mechanical ventilation, the chronically infected tracheal secretions did not contain any ESBLE. The epidemic strain was found in the environment, but only very close to colonized and/or infected patients. The mode of transmission of the ESBLE during outbreaks has rarely been identified. However, it is argued that cross-transmission occurs mainly by staff handling [31]. Even if this is the most frequent mode of acquisition of ESBLE, our data indicate that such strains may persist in the immediate environment of patients, and that indirect transmission by inert equipment cannot be excluded. Cross-transmission in this ICU was certainly enhanced by the intense nursing contact required by patients of hall A, whereas patients of hall B only needed conventional medical care. The epidemic strain had been probably established in this ICU for at least several weeks prior to the study, since patient D, who carried the strain on his arrival, had been already hospitalized in the same ICU 2 months before. Spontaneous loss of ESBLE did not occur outside of the hospital, in this case. Finally, our findings are consistent with the observation that the propagation of SHV-4 β-lactamase is usually related to strain rather than to plasmid dissemination [15–17]. On the other hand, strains isolated from patient H (ATB2, P2, E2) were of the same ribotype as that of the epidemic strain found in this unit (halls A and B) in 1993 [20]. Thus, using genetic tools, we revealed here the replacement of one epidemic strain by another in the same unit.

The other goal of the study was to analyse the characteristics of the patients who carried an ESBLE during the study period, in comparison with patients who did not. The low number of patients with an ESBLE included in the study ($n = 7$) did not allow us to identify precisely the risk factors for ESBLE acquisition. Nevertheless, in agreement with many studies, the length of hospitalization appeared to be a major risk factor for ESBLE acquisition [21]. The number of surgical operations was also greater in the colonized and/or infected group than in the control one. In contrast, no difference was noted for severity of illness at admission and underlying diseases, intensity of care and invasive procedures. The contribution of different practices to transmission could

not be measured [30]. However, the increased rate of surgical interventions might be considered as an indication that colonized and/or infected patients belonged to the most critically-ill ICU population. Previous studies identified antimicrobial exposure as a risk factor for infections with antimicrobial-resistant enterobacteriaceae [21]. In our study, where only two patients were infected with ESBLE, treatment with broad-spectrum β-lactam antibiotics seemed to be a risk factor for ESBLE infection.

In conclusion, this study confirms the importance of typing suspected epidemic strains in order to understand the natural history of ESBLE outbreaks. One clonal strain was responsible for the ICU-acquired cases in hall A. The risk of acquiring the ESBLE increased post-operatively and with the length of stay in the ICU. Starting at the beginning of 1995, nursing procedures (handwashing, single-use equipment and waste control) were intensified or modified, particularly for patients at high risk; colonized and/or infected patients were isolated and cohort nursing was implemented. Intestinal decontamination combined with these procedures avoided ward closure and led to the eradication of the epidemic strain.

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