Eur. J. Clin. Microbiol. Infect.Dis., November 1990, p. 797–803
0934-9723/90/110 0797-07 $ 3.00/0

Outbreak of Nosocomial Infections due to *Klebsiella pneumoniae* Producing SHV-4 Beta-Lactamase

G. Arlet[1]*, M. J. Sanson-le Pors[1], M. Rouveau[1], G. Fournier[2], O. Marie[3], B. Schlemmer[3], A. Philippon[4]

One hundred and fifty-four clinical isolates of *Klebsiella pneumoniae* resistant to broad-spectrum cephalosporins, aztreonam and amikacin were responsible for an outbreak of nosocomial infections lasting eight months in a university hospital in Paris. This outbreak occurred in the intensive care unit (39 patients), haematology units (8 patients) and surgical and medical units (11 patients). Antibiotic resistant strains were isolated from the urinary tract (48 %), wound and drainage fluids (21 %), respiratory tract (14 %), blood (12 %) and stools (5 %). High resistance to oxyimino-beta-lactams was mediated by a plasmid-encoded beta-lactamase with an isoelectric point of 7.8 (SHV-4). This CAZ-type enzyme conferred a higher level of resistance to ceftazidime and aztreonam (geometric mean MIC 135 mg/l) than to cefotaxime (geometric mean MIC 14 mg/l). All isolates were of the same biotype (weakly urease positive and no sucrose fermentation). Eight *Klebsiella pneumoniae* strains isolated in different units and at different times of the outbreak were of the same serotype, had common plasmid patterns and harboured a large self-transferable plasmid of about 180 kilobases encoding resistance to penicillins, oxyimino-beta-lactams, aminoglycosides, tetracycline and trimethoprim. These eight large plasmids had indistinguishable *Eco*RI restriction patterns. These results suggest that a single strain of *Klebsiella pneumoniae* was responsible for this outbreak.

Cephalothin and gentamicin resistant clinical isolates of *Klebsiella pneumoniae* have been responsible for outbreaks of nosocomial infections in the seventies (1, 2). Recently, ten years after the introduction of broad-spectrum cephalosporins and amikacin, several outbreaks of infection caused by *Klebsiella pneumoniae* simultaneously resistant to both these groups of antibiotics have been reported (3–5). These multiresistant isolates produce the novel plasmid-mediated beta-lactamses CTX-1 (6), SHV-2 (7) and SHV-3 (5), which are able to hydrolyze oxyimino-beta-lactams. Hybridization tests and nucleotide or peptide sequencing has demonstrated that they are derived from TEM-2 (CTX-1 or TEM-3) or SHV related enzymes (8–10). One or more amino-acid substitutions create new interactions between the enzyme and the substrate, allowing efficient hydrolysis of oxyimino-beta-lactams (9, 11). These extended spectrum beta-lactamases are CTX-type enzymes because they confer a higher level of resistance to cefotaxime and ceftriaxone than to ceftazidime and aztreonam (3, 7).

[1]Laboratoire de Microbiologie, Hôpital Saint-Louis, 1 Avenue Claude Vellefaux, 75010 Paris, France.
[2]Laboratoire de Microbiologie, Faculté de Médecine Cochin-Port Royal, Paris, France.
[3]Service de Réanimation, Hôpital Saint-Louis, Paris, France.
[4]Unité d'Ecologie Bactérienne, Institut Pasteur, Paris, France.

More recently, multiresistant *Klebsiella pneumoniae* belonging to the serotype K25 were isolated in clusters in five hospitals in the Paris area (12). A new extended spectrum beta-lactamase, designated SHV-4 (and also CAZ-5) (13), with a pI of 7.8 was characterized. This CAZ-type enzyme conferred a higher level of resistance to ceftazidime and aztreonam than did SHV-2 and SHV-3 (12). Between September 1987 and May 1988, we observed an outbreak of nosocomial infections caused by strains of multiresistant *Klebsiella pneumoniae* in our hospital. This paper describes bacteriological, genetic and epidemiological aspects of this outbreak.

Materials and Methods

Identification of Bacterial Isolates. Between September 1987 and May 1988, 154 clinical isolates of multiresistant *Klebsiella pneumoniae* were recovered from 58 patients in the medical and surgical intensive care unit, haematology units, and surgical and medical units. Isolates were identified using the API 20E system (API, France) and the first isolate from each patient was biotyped with the API 50CH system. The biotypes of these first isolates were read after 18 h at 37 °C. Capsular serotyping was done by P. Bouvet (Institut Pasteur, France).

Antibiotic Susceptibility Testing. Susceptibility testing was performed on Mueller-Hinton agar plates by the disk diffusion technique (Diagnostics Pasteur, France). The presence of

extended spectrum beta-lactamase was detected using the double-disk synergy test (14). MICs of various beta-lactams, of cefotaxime and ceftazidime in the presence of clavulanic acid (2 mg/l) or sulbactam (2 mg/l), and of gentamicin and amikacin, were determined for the first isolate from each patient. Serial two-fold dilutions of antibiotics in Mueller-Hinton agar and inocula of about 10^5 cfu per spot were used; inocula were delivered by a multipoint inoculator. MICs were read after incubation for 18 h at 37 °C and defined as the lowest antibiotic concentration at which no growth was visible.

Transfer of Oxyimino-Beta-Lactams Resistance. Escherichia coli K12 strain J53-2 *met* F63 *pro* 22 rif was used as the recipient for conjugation of the plasmids encoding the extended spectrum beta-lactamase. *Escherichia coli* transconjugants were selected on plates containing Mueller-Hinton agar supplemented with ceftazidime (2 mg/l) and rifampicin (250 mg/l). The frequencies of transfer are expressed as a fraction of the number of donor cells.

Beta-Lactamase Assay. Analytical isoelectric focusing was performed on polyacrylamide gels (15) with crude cell-free sonic extracts. Beta-lactamase activity was detected by the iodometric method with ceftriaxone (20 mg/100 ml of gel) as substrate (5), and then by the classic chromogenic nitrocefin test. CTX-1 (pCFF04, pI 6.3), SHV-2 (pBP60-1, pI 7.6), and SHV-4 (pUD21, pI 7.8) were used as pI markers. Vmax was measured as reported elsewhere by a microacidimetric method (5). One unit of beta-lactamase (u) was defined as that amount of enzyme which hydrolyzed 1 µmol of benzylpenicillin per min at pH 7.0 and 37 °C. Inhibition of beta-lactamase activity by clavulanic acid was determined as elsewhere reported (5).

Plasmid Analysis. Plasmid DNA was extracted by the method of Takahashi and Nagano (16) from eight strains isolated from eight patients at different times of the outbreak and in different units. Plasmid DNA was digested with *Eco*RI under the conditions recommended by the supplier (Bethesda Research Laboratories, USA). Digested and non-digested DNA were analysed by electrophoresis through a 0.7 % agarose gel for 3 h at 7 V/cm in Tris acetate buffer.

Epidemiological Investigation. The Saint-Louis Hospital is a university hospital with about 800 beds, including haematology, immunology, bone marrow transplantation, dermatology, endocrinology, nephrology, urology, medical, surgical and intensive care units. The orthopaedic surgery unit is in a separate building. The surgical and medical intensive care unit is composed of two subunits: an acute care ward on the ground floor, and an intermediate care ward on the first floor.

Colonisation and infection were not differentiated. For patients in the medical and surgical intensive care unit, data was recorded on the duration of stay and mechanical ventilation, the simplified acute physiological score (SAPS) (17), urinary tract catheterisation and antimicrobial therapy. We compared the infected patients to all patients and to a control group of patients with mechanical ventilation and urinary tract catheterisation hospitalized during the outbreak but without having been infected.

Results

Bacteriological Analysis. The 144 clinical isolates of *Klebsiella pneumoniae* were of a particular biotype as shown by the API 20E system: they had a weak urease activity and did not ferment sucrose. The API 50CH system showed the first isolates of each patient to be identical. This biotype was similar to the biotype 'a' described by Richard (18). Eight strains of *Klebsiella pneumoniae* isolated in different units and at different times of the outbreak had the same serotype K25. All isolates exhibited the same phenotypic resistance pattern as determined by the disk diffusion method. They were resistant to penicillins, oxyimino-beta-lactams, aminoglycosides (except gentamicin), tetracycline, chloramphenicol, trimethoprim and fluoroquinolones. The ceftazidime and aztreonam inhibition zone diameters (≤ 12 mm) were smaller than those observed for cefotaxime

Table 1: Susceptibility of initial clinical isolates of *Klebsiella pneumoniae* producing SHV-4 beta-lactamase to beta-lactam antibiotics, gentamicin and amikacin.

Antimicrobial agent	Number of isolates with the following MIC (mg/l)												
	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	> 128
Carbenicillin													58
Piperacillin													58
Cefotaxime							9	11	24	12		2	
Cefotaxime + CA[a]	7	45	5	1									
Cefotaxime + SB[a]		34	1	2	7	1	6	7					
Ceftazidime											15	22	21
Ceftazidime + CA[a]			29	20	9								
Ceftazidime + SB[a]				34		3	6	15					
Aztreonam											1	31	26
Moxalactam				12	24	17	3	2					
Cefotetan			28	27		1	2						
Imipenem	24	33	1										
Gentamicin					28	28	1	1					
Amikacin								3	53	1			

[a]Clavulanic acid (CA) and sulbactam (SB) were each used at a concentration of 2 mg/l.

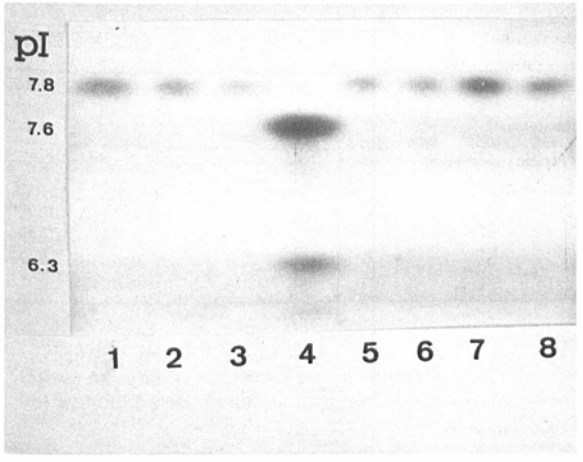

Figure 1: Analytical isoelectric focusing of SHV-4 beta-lactamase produced by *Klebsiella pneumoniae*. Lanes 1–3: Clinical isolates of *Klebsiella pneumoniae* producing SHV-4 beta-lactamase; lane 4: CTX-1 (pCFF04, pI 6.3) and SHV-2 (pBP60-1, pI 7.6); lane 5: SHV-4 (pUD21, pI 7.8); lanes 6–8: *Escherichia coli* K12 J53-2 transconjugants.

Table 2: Comparative properties of the beta-lactamases derived from the *Klebsiella pneumoniae* SLK-01 strain.

Property	Beta-lactamase[a]	
	SHV-4 (pUD21)	SLK-01
pI	7.8	7.8
Rate of hydrolysis[b]		
Amoxicillin	185	214
Carbenicillin	33	40
Cephaloridine	321	400
Cefotaxime	71	104
Ceftazidime	19	21
Aztreonam	< 1	< 1
Inhibition[c] by clavulanate (1 μM)	78	83

[a]Sonicated extracts of *Escherichia coli* K12 J53-2 *met* F63 *pro* 22 rif (plasmid number in parenthesis).

[b]Expressed as Vmax relative to that of benzylpenicillin set at 100.

[c]Percent inhibition with benzylpenicillin set at 100.

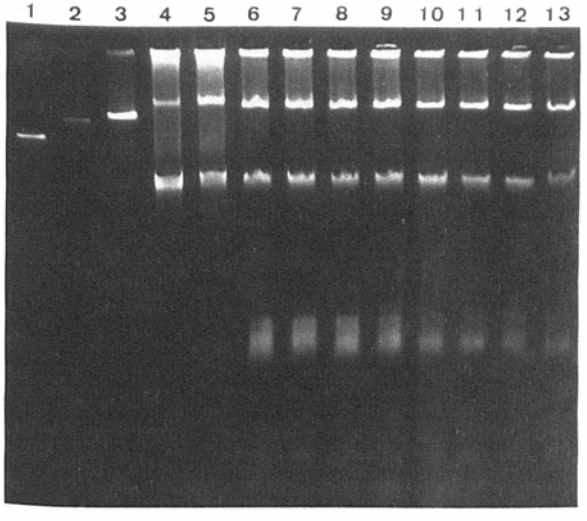

Figure 2: Plasmid patterns of clinical isolates of *Klebsiella pneumoniae* producing SHV-4 beta-lactamase. Lane 1: pIP 135-1 (70.4 Kb); lane 2: pIP 112 (100.5 Kb); lane 3: pIP 173 (125.8 Kb); lanes 4 and 5: *Escherichia coli* K12 J53-2 transconjugants; lanes 6–13: clinical isolates of *Klebsiella pneumoniae* that produce SHV-4 beta-lactamase.

(16–22 mm) (CAZ resistant phenotype). Some of the strains isolated in May 1988, late in the study, showed decreased susceptibility to moxalactam and cephamycins. The MICs (Table 1) of ceftazidime and aztreonam (geometric means ≥ 135 mg/l) were higher than that of cefotaxime (geometric mean 14 mg/l). All strains remained susceptible to imipenem, and the majority of the isolates was susceptible to moxalactam and cefotetan. Two mg/l clavulanic acid was three times more efficient than 2 mg/l sulbactam in restoring the activity of cefotaxime and ceftazidime (geometric mean MICs 0.12 versus 0.41 mg/l and 0.39 versus 1.36 mg/l respectively).

A single band of beta-lactamase activity with a pI of 7.8 was detected in each first isolate and their transconjugant by the iodometric procedure using ceftriaxone as substrate (Figure 1). One additional band with a pI of 7.6 was identified by the nitrocefin method. This band corresponds to the chromosomal beta-lactamase of *Klebsiella pneumoniae*. The beta-lactamase produced by the first strain isolated had the same substrate profile as SHV-4 (pUD21) (Table 2). The eight strains of *Klebsiella pneumoniae* isolated in different units had a common plasmid profile (Figure 2). A large plasmid of about 180 kilobases (kb) could be transferred by conjugation into *Escherichia coli* J53-2 (Figure 2) with a high frequency (10^{-5}). The presence of these plasmids correlated with resistance to penicillins, oxyimino-beta-lactams, aminoglycosides (except gentamicin), tetracycline and trimethoprim. Plasmid DNA was prepared from each transconjugant and digested with *Eco*RI. The eight plasmids had indistinguishable restriction fragment patterns (Figure 3).

Epidemiological Investigation. The first isolate of extended spectrum beta-lactamase-producing *Klebsiella pneumoniae* was isolated in September 1987 from a patient in the intensive care unit. During the following weeks (Figure 4) multiresistant *Klebsiella pneumoniae* were isolated from other patients in the intensive care unit and also from patients in

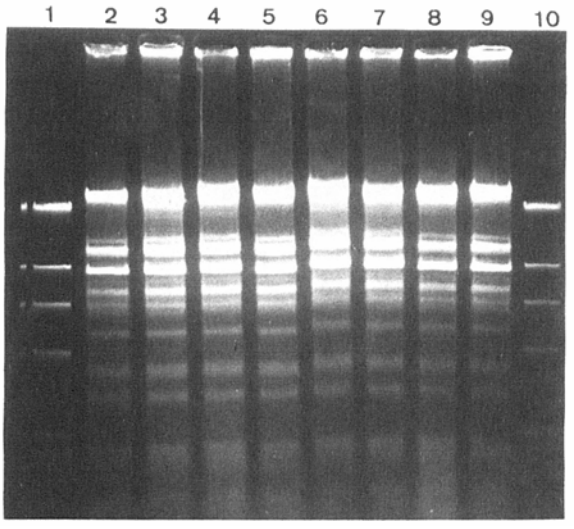

Figure 3: *Eco*RI restriction endonuclease digestion of plasmids from *Escherichia coli* K12 J53-2 transconjugants. Lanes 1 and 10: λ digested by *Hin*dIII; lanes 2–9: plasmid DNA from transconjugants of different *Klebsiella pneumoniae*.

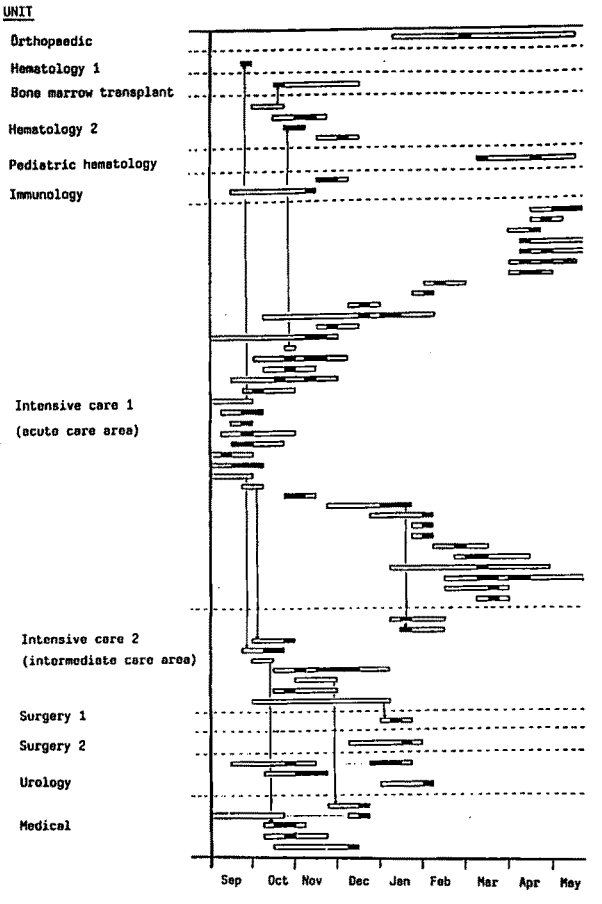

Figure 4: Distribution over time and in different units of patients infected by *Klebsiella pneumoniae* producing SHV-4 beta-lactamase. Blank bar: duration of stay; solid bar: duration of infection.

other units who had recently been transferred from the intensive care unit. The strain spread, essentially to the intensive care unit, but also to the haematological, medical and surgical units. After February 1988, *Klebsiella pneumoniae* were only isolated in the intensive care unit, with one exception.

The analysis of sites of infection (Table 3) showed that 42 % of the multiresistant *Klebsiella pneumoniae* were isolated from the urinary tract (32 patients of whom 29 had urinary tract catheters) and 28 % from surgical wounds (15 patients). All multiresistant strains isolated from the respiratory tract (12 %) were from patients on mechanical ventilation. *Klebsiella pneumoniae* were recovered from blood cultures in 11 patients; in two of them, the blood was the only site of a positive culture. *Klebsiella pneumoniae* were also isolated from the stools in four neutropenic patients; two of them had positive blood cultures. Finally, multiresistant *Klebsiella pneumoniae* were the cause of death in five patients.

Thirty-five percent of patients (23/65) hospitalized in the intensive care unit for surgical care during the outbreak were infected by the multiresistant *Klebsiella pneumoniae*. Only 7 % of medical patients (16/220) were infected. In surgical patients, the major sites of infection were wounds and drainage fluids; these were also the sites of first isolation in nine patients. On the other hand, the first and major site of infection in medical patients was the urinary tract (50 %). The risk factors for infection or colonization were analysed. Only the duration of stay and of

Table 3: Sites of infection of *Klebsiella pneumoniae* producing SHV-4 beta-lactamase.

	Urine	Respiratory tract	Wound and drainage fluid	Stool	Blood
Number of isolates	65	18	44	5	22
Number of patients	32	12	15	4	11
Number of patients with another site of infection	9	6	9	2	9
Number of patients with septicaemia	3	1	3	2	11

Table 4: Epidemiological analysis of the outbreak of infection in the intensive care unit due to *Klebsiella pneumoniae* producing SHV-4 beta-lactamase.

	Total no. of patients		Control group[a]		Infected group	
	Medical	Surgical	Medical	Surgical	Medical	Surgical
Number of patients	220	65	54	25	16	23
Mean age (year)	55	55	61	58	55	62
Mean SAPS	11	12	13	14	14	16
Mean duration of stay (days)	8	14	20	15	44	57
Mean duration of MV (days)	8	14	15	13	27	43

SAPS: simplified acute physiological score. MV: mechanical ventilation.

[a]Group of patients with mechanical ventilation (≥ 4 days) and urinary tract catheterisation hospitalized during the outbreak, but without having been infected.

mechanical ventilation differed markedly between infected and non-infected groups (Table 4). The delay before infection or colonization was extremely variable: between 2 and 114 days after admission (mean 14 days).

The multiresistant strains were isolated in 39 intensive care patients. Two patients received no antibiotics, and three received antimicrobial agents usually inactive against *Enterobacteriaceae*. The other 34 patients received either beta-lactams (ceftazidime, ceftriaxone, piperacillin or amoxicillin) or aminoglycosides (amikacin, tobramycin, netilmicin) or fluoroquinolones or cotrimoxazole; most of them (60 %) received two antibiotics simultaneously.

Discussion

The Saint-Louis Hospital experienced an outbreak of nosocomial infections caused by *Klebsiella pneumoniae* producing an extended spectrum beta-lactamase. All the strains of *Klebsiella pneumoniae* isolated in our hospital were of a particular biotype (weakly urease positive and no sucrose fermentation). The high level of resistance to ceftazidime and aztreonam of these *Klebsiella pneumoniae*, associated with the characteristic biotype allowed easy detection of these strains. This is the first description of an outbreak of nosocomial infections due to *Klebsiella pneumoniae* producing a CAZ-type extended spectrum beta-lactamase. As reported for the other extended spectrum beta-lactamases (3, 5, 6, 7, 14, 19, 20), the beta-lactamase inhibitors clavulanic acid and sulbactam showed a synergistic effect in vitro in combination with cephalosporins. Despite the high level of resistance to beta-lactams conferred by this beta-lactamase, the use of combinations of amoxicillin with clavulanic acid or of piperacillin with sulbactam led to the cure of some of the urinary tract infections where there was no catheterisation. Most of the strains isolated late in our study showed decreased susceptibility to cephamycins and moxalactam, and clavulanic acid and especially sulbactam were less effective in restoring the activity of the oxyimino-beta-lactams. As previously suggested (19), a decrease in permeability might interfere with inhibitor efficacy, and it may be that sulbactam is more affected than clavulanic acid by permeability changes. Our strains were also resistant to aminoglycosides other than gentamicin (probably due to AAC (6') IV) and had a high level of resistance to pefloxacin. Treatment of serious infections was limited to imipenem alone or combined with gentamicin.

Our strains produced an extended spectrum beta-lactamase with a pI of 7.8 and a substrate profile similar to that of SHV-4 beta-lactamase (12) which was simultaneously observed in strains from four other Parisian hospitals (12). The first strains isolated in each hospital belonged to serotype K25 and had the same characteristic biotype as our strains. The SHV-4 beta-lactamase is probably derived from SHV-3 beta-lactamase (5) by only one amino-acid substitution between their primary structures (10, 13).

In common with the *Klebsiella pneumoniae* producing CTX-1 (TEM-3) responsible for previous outbreaks (3, 4), our strains were resistant to aminoglycosides (except gentamicin). The association of extended spectrum beta-lactamase and resistance to amikacin is probably a major factor in the dissemination of these strains (4), especially when broad-spectrum cephalosporins and amikacin exert a selective pressure. In our hospital vancomycin, ceftazidime and amikacin were the most widely used antibiotics in the intensive care unit before and during the period of the outbreak (P. Faure, personal communication).

Although the outbreaks of nosocomial infections due to CTX-1-producing *Enterobacteriaceae* (3, 4, 20) were the result of dissemination of a transmissible plasmid of 85 Kb to *Klebsiella pneumoniae* strains of various different biotypes and serotypes and six

species of *Enterobacteriaceae*, during the outbreak in our hospital the SHV-4 beta-lactamase was produced only by *Klebsiella pneumoniae* strains. All these strains were of the same biotype. Moreover, eight strains isolated in different units were of the same serotype and had the same plasmid profile. Their transconjugants had acquired a large plasmid showing the same *Eco*RI restriction pattern. These results strongly suggest that a single strain of *Klebsiella pneumoniae* was responsible for the outbreak in our hospital.

Unlike in the outbreak of nosocomial infections due to CTX-1 *Klebsiella pneumoniae* (3), there was rapid dissemination of multiresistant *Klebsiella pneumoniae* from the intensive care unit to the haematology units, and surgical and medical units in our outbreak, despite the absence of virulence factors such as aerobactin or the mucoid phenotype (21, 22, V. Vernet, personal communication).

As already reported (3, 4), *Klebsiella pneumoniae* producing extended spectrum beta-lactamases have been isolated from patients with nosocomial infections. In our study urinary infections were the major source of these strains, associated with urinary tract catheterisation in the medical patients. In surgical patients wound and drainage fluid appeared to be the major source of microorganisms, consistent with the findings of an international survey of hospital-acquired infection (23). Urinary tract catheterisation did not systematically cause infection. During the period of the outbreak 79 patients in the intensive care unit had mechanical ventilation and urinary tract catheterisation and were not infected. Nevertheless, the major risk factors were the duration of stay, of mechanical ventilation and of urinary tract catheterisation. Moreover, as shown elsewhere (1, 3), intestinal colonisation preceded infection. It is thus necessary to identify fecal carriers of multiresistant *Klebsiella pneumoniae* (1) in order to control similar outbreaks by aseptic practices and intestinal decontamination (24).

These extended spectrum beta-lactamases are now a worldwide problem. Various epidemiological features of these strains have been observed particularly in developing countries where these beta-lactamases are found especially in *Salmonella* species isolated from children (25).

Acknowledgements

We would like to thank N. Vittecoq for secretarial assistance, P. Bouvet for capsular serotyping, I. Casin and F. Morinet for valuable discussions and Professor Y. Perol for her constant support and interest for this project.

References

1. **Casewell MW, Phillips I:** Aspect of the plasmid-mediated antibiotic resistance and epidemiology of *Klebsiella* species. American Journal of Medicine 1981, 70: 459–462.
2. **Sadowski PL, Peterson BC, Gerding DN, Cleary PP:** Physical characterization of ten R plasmids obtained from an outbreak of nosocomial *Klebsiella pneumoniae* infections. Antimicrobial Agents and Chemotherapy 1979, 15: 616–624.
3. **Brun-Buisson C, Legrand P, Philippon A, Montravers F, Ansquer M, Duval J:** Transferable enzymatic resistance to third-generation cephalosporins during nosocomial outbreak of multiresistant *Klebsiella pneumoniae*. Lancet 1987, ii: 302–306.
4. **Sirot J, Chanal C, Petit A, Sirot D, Labia R, Gerbaud G:** *Klebsiella pneumoniae* and other *Enterobacteriaceae* producing novel plasmid-mediated beta-lactamases markedly active against third-generation cephalosporins: epidemiologic studies. Reviews of Infectious Diseases 1988, 10: 850–859.
5. **Jarlier V, Nicolas MH, Fournier G, Philippon A:** Extended broad-spectrum beta-lactamases conferring transferable resistance to newer beta-lactam agents in *Enterobacteriaceae*: hospital prevalence and susceptibility patterns. Reviews of Infectious Diseases 1988, 10: 867–878.
6. **Sirot D, Sirot J, Labia R, Morand A, Courvalin P, Darfeuille-Michaud A, Perroux R, Cluzel R:** Transferable resistance to third-generation cephalosporins in clinical isolates of *Klebsiella pneumoniae*. Identification of CTX-1, a novel beta-lactamase. Journal of Antimicrobial Chemotherapy 1987, 20: 323–334.
7. **Kliebe C, Nies BA, Meyer JF, Tolxdorff-Neutzling RM, Wiedemann B:** Evolution of plasmid-coded resistance to broad-spectrum cephalosporins. Antimicrobial Agents and Chemotherapy 1985, 28: 302–307.
8. **Barthélémy M, Péduzzi J, Ben Yaghlane H, Labia R:** Single amino acid substitution between SHV-1 beta-lactamase and cefotaxime-hydrolyzing SHV-2 enzyme. FEBS Letters 1988, 231: 217–220.
9. **Sougakoff W, Goussard S, Courvalin P:** The TEM-3 beta-lactamase, which hydrolyzes broad-spectrum cephalosporins, is derived from the TEM-2 penicillinase by two amino acid substitutions. FEMS Microbiology Letters 1988, 56: 343–348.
10. **Nicolas MH, Jarlier V, Honoré N, Philippon A, Cole S:** Molecular characterization of the gene encoding SHV-3 beta-lactamase responsible for transferable cefotaxime resistance in clinical isolates of *Klebsiella pneumoniae*. Antimicrobial Agents and Chemotherapy 1989, 33: 2096–2100.
11. **Philippon A, Labia R, Jacoby G:** Extended-spectrum beta-lactamases. Antimicrobial Agents and Chemotherapy 1989, 33: 1131–1136.
12. **Buré A, Legrand P, Arlet G, Jarlier V, Paul G, Philippon A:** Dissemination in five French hospitals of *Klebsiella pneumoniae* serotype K25 harbouring a new transferable enzymatic resistance to third generation cephalosporins and aztreonam. European Journal of Clinical Microbiology and Infectious Diseases 1988, 7: 780–782.
13. **Péduzzi J, Barthélémy M, Tiwari K, Mattioni D, Labia R:** Structural features related to hydrolytic activity against ceftazidime of plasmid-mediated SHV-type CAZ-5 beta-lactamase. Antimicrobial Agents and Chemotherapy 1989, 33: 2160–2163.

14. **Legrand P, Fournier G, Buré A, Jarlier V, Nicolas MH, Decré D, Duval J, Philippon A:** Detection of extended broad-spectrum beta-lactamases in *Enterobacteriaceae* in four French hospitals. European Journal of Clinical Microbiology and Infectious Diseases 1989, 8: 527–529.
15. **Matthew M, Harris AM, Marshall H, Ross GW:** The use of analytic isoelectric focusing for detection and identification of beta-lactamases. Journal of General Microbiology 1975, 88: 169–178.
16. **Takahashi S, Nagano Y:** Rapid procedure for isolation plasmid DNA and application to epidemiological analysis. Journal of Clinical Microbiology 1984, 20: 608–613.
17. **Le Gall JR, Loirat Ph, Alperovitch A:** Simplified acute physiological score for intensive care patients. Lancet 1983, ii: 741.
18. **Richard C:** Bacteriologie et épidémiologie des espèces du genre *Klebsiella*. Bulletin de l'Institut Pasteur 1982, 80: 127–145.
19. **Gutmann L, Ferré B, Goldstein FW, Rizk N, Pinto-Schuster E, Acar JF, Collatz E:** SHV-5, a novel SHV-type beta-lactamase that hydrolyzes broad-spectrum cephalosporins and monobactams. Antimicrobial Agents and Chemotherapy 1989, 33: 951–956.
20. **Kitzis MD, Billot-Klein D, Goldstein FW, Williamson R, Tran Van Nhieu G, Carlet J, Acar JF, Gutmann L:** Dissemination of the novel plasmid-mediated beta-lactamase CTX-1, which confers resistance to broad-spectrum cephalosporins, and its inhibition by beta-lactamase inhibitors. Antimicrobial Agents and Chemotherapy 1988, 32: 9–14.
21. **Nassif X, Sansonetti PJ:** Correlation of the virulence of *Klebsiella pneumoniae* K1 and K2 with the presence of a plasmid encoding aerobactin. Infection and Immunity 1986, 54: 603–608.
22. **Nassif X, Fournier JM, Arondel J, Sansonetti PJ:** Mucoid phenotype of *Klebsiella pneumoniae* is a plasmid-encoded virulence factor. Infection and Immunity 1989, 57: 546–552.
23. **Mayon-White RT, Ducel G, Kereselidze T, Tikorimov E:** An international survey of the prevalence of hospital-acquired infection. Journal of Hospital Infection 1988, 11: 43–48.
24. **Brun-Buisson C, Legrand P, Rauss A, Richard C, Montravers F, Besbes M, Meakins JL, Soussy CJ, Lemaire F:** Intestinal decontamination for control of nosocomial multiresistant gram-negative bacilli. Annals of Internal Medicine 1989, 110: 873–881.
25. **Philippon A, Ben Redjeb S, Fournier G, Ben Hassen A:** Epidemiology of extended spectrum beta-lactamases. Infection 1989, 17: 347–354.