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Hospital dispersion of *Staphylococcus epidermidis* isolates resistant to a fluoroquinolone, pefloxacin

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SUMMARY

Since 1985, nosocomial infections have been frequently treated with a new fluoroquinolone, pefloxacin, at the Cardiological and Neurological Hospital in Lyon. From 1986 to 1988, the incidence of resistance of clinical *Staphylococcus epidermidis* strains to pefloxacin increased from 31 to 57%. Dispersion of these resistant strains in the hospital environment was recognized when they were detected on 22% of staff members' fingers (139 samples were investigated) and in 28% of the environmental samples (180 were investigated). There was an association between carriage rate and work place. Most of the pefloxacin-resistant *S. epidermidis* were resistant to oxacillin, gentamicin, erythromycin, co-trimoxazole and fosfomycin. Intensive use of pefloxacin selected multiresistant *S. epidermidis* which became ubiquitous in the hospital environment.

INTRODUCTION

Most infections caused by coagulase-negative staphylococci (CNS) are hospital-acquired and are due to strains resistant to multiple antibiotics (1). The multiresistant phenotype is defined as one that encompasses resistance to methicillin and at least three other unrelated classes of antibiotics, such as aminoglycosides, macrolides and co-trimoxazole (2). Among the CNS, multiresistant *S. epidermidis* is the species primarily responsible for nosocomial infections (3).

Pefloxacin is a fluoroquinolone with a broad spectrum of activity against pathogens including staphylococci and Gram-negative bacteria (4, 5). Since 1985, it has been used intensively in the Cardiological and Neurological Hospital of Lyon for the treatment of severe infections. From 1986 to 1988, the proportion of clinical isolates of *S. epidermidis* resistant to pefloxacin increased from 31 to 57%. In 1988, we investigated the hospital distribution of pefloxacin resistant *S. epidermidis* strains isolated from patients, staff and the hospital environment. Pefloxacin-resistant strains were isolated from all sites and appeared to have a multiresistant phenotype.

MATERIALS AND METHODS

The Neurological and Cardiological Hospital in Lyon is divided into two buildings of 437 and 474 beds respectively. In 1988, 10170 patients were hospitalized in the neurological units, and 10791 in the cardiological units. Patients operated on with circulation bypass received 48-h antibiotic prophylaxis with cefazolin and netilmicin, or, for heart transplant operations only, with ceftriaxone and pefloxacin; those for neurological surgery received cefamandole per-operative prophylaxis. From all the routine clinical samples, 2646 CNS were isolated in 1988. Of these 313 were identified as *S. epidermidis*, 106 as species other than *S. epidermidis* and 2227 were left undetermined. A total of 5·5 kg of pefloxacin were prescribed in 1988 to treat 520 patients infected with Gram-positive or Gram-negative bacteria.

Pefloxacin-resistant *S. epidermidis* were investigated in the staff and in the hospital environment. In each building, four different groups of units were studied: medical units, surgery, intensive care, and operating theatre. Resistant strains were sought in the linen room where patients' bed-sheets are sorted before being laundered. The control group consisted of kitchen and administration employees who had no direct contact with the hospital environment and had not received previous antibiotic treatment. Control samples were also taken from the employees' environment.

Fingerprint plates were obtained from 139 staff members and from 33 in the control group. Blood agar plates (Bio-Mérieux, Charbonnières, France) were used for all primary platings. One hundred and eighty hospital environment samples and 33 control samples were taken using Count-Tact plates (Bio-Mérieux).

Primary plates were incubated overnight at 37 °C. Pefloxacin-resistant strains were isolated by replica plating on brain-heart agar plates (Bio-Mérieux) supplemented with 16 μg/ml of pefloxacin. Supplemented plates were incubated for 48 h at 37 °C. Gram-positive pefloxacin-resistant cocci were subcultured for further identification testing. CNS were identified using the API Staph gallery (API System, Montalieu-Vercieu, France).

Antibiotic susceptibility was determined by the agar diffusion method on Mueller–Hinton medium (Bio-Mérieux) with commercial disks of penicillin G (6 μg), oxacillin (5 μg), gentamicin (15 μg), chloramphenicol (30 μg), tetracycline (30 IU), erythromycin (15 IU), clindamycin (15 μg), pristinamycin (15 μg), rifampicin (30 μg), co-trimoxazole (1·25 and 23·75 μg), fosfomycin (50 μg), fusidic acid (10 μg) and vancomycin (30 μg). The sizes of the inhibition zones were interpreted according to the recommendations of the Comité Français de l'Antibiogramme (6). Zones for pefloxacin-susceptible strains was equal to or more than 22 mm in diameter and for resistant strains less than 16 mm; the corresponding minimal inhibitory concentrations were 1 and 4 μg/ml respectively. Intermediate strains with zones between 16–22 mm in diameter were considered resistant to pefloxacin.

RESULTS

In 1988, 176 (57%) of 313 *S. epidermidis* isolates from routine clinical sources and 37% of the isolates clinically considered to be responsible for infections were

Table 1. *Antibiotic resistance phenotype of* S. epidermidis *strains, susceptible or resistant to pefloxacin*

Antibiotics	Patient isolates (%) Pefloxacin Susceptible ($n = 133$)	Patient isolates (%) Pefloxacin Resistant ($n = 176$)	Environmental isolates (%) Pefloxacin resistant ($n = 82$)
Benzyl-penicillin	77	99	96
Oxacillin	32	95	87
Gentamicin	28	92	71
Chloramphenicol	10	46	24
Tetracyclines	23	22	32
Erythromycin	33	97	92
Pristinamycin	4	12	2·5
Rifampin	10	64	49
Co-trimoxazole	20	94	71
Fosfosmycin	19	85	89
Fusidic acid	10	20	1·2
Vancomycin	0	0	0

Table 2. *Site and isolation frequency of pefloxacin-resistant* S. epidermidis *isolates*

	Fingerprints		Environmental samples	
	*n/s**	%	*n/s*	%
Intensive care units	8/28	29	23/55	42
Operating theatres	2/36	5·5	3/40	7·5
Surgical units	9/29	31	11/42	26
Medical units	7/38	18	11/39	28
Linen room	4/8		3/4	
Total	30/139	22	51/180	29
Control group	1/33	3	0/34	0

* n, total number of samples with pefloxacin-resistant *S. epidermidis* isolated; s, total number of samples.

resistant to pefloxacin. They had a multi-resistant phenotype compared with that of pefloxacin-susceptible *S. epidermidis* isolates (Table 1). The antibiotic resistance patterns of the pefloxacin-resistant CNS, which were not identified at the species level, were similar to those of *S. epidermidis* (data not shown).

Pefloxacin-resistant *S. epidermidis* were searched for in 319 samples from the hands of hospital staff and from surfaces and were detected in 22 and 28% respectively (Table 2). Recovery of resistant isolates from environmental samples was associated with the finger carriage of staff working in the same ward. The resistance patterns of these isolates was closely similar to that of patient isolates (Table 1). In the control group, they were not detected in any environmental samples and in only 1 out of 33 fingerprints.

Resistant isolates were found more frequently in the intensive care units and surgical units of both hospital buildings, in the medical cardiological units and in the linen room (Table 3). Resistant strains were rarely isolated in the neurological

Table 3. *Isolation frequency of pefloxacin-resistant* S. epidermidis *in the Cardiological and Neurological Hospital, Lyon*

	Cardiological Hospital		Neurological Hospital		Total	
	n/*s**	%	*n*/*s**	%	*n*/*s**	%
Intensive care units	9/44	20	22/39	54	31/83	37
Operating theatres	0/36	0	5/40	12·5	5/76	7
Surgical units	6/34	18	14/37	38	20/71	28
Medical units	17/40	42·5	1/37	3	18/77	23
Linen room					7/12	
Total	32/154	21	42/153	27	81/319	25
Control group					1/67	1·5

* *n*, total number of samples with pefloxacin-resistant *S. epidermidis* isolated; *s*, total number of samples.

medical units where very few patients were treated for infections ($P < 0{\cdot}001$). These units accounted for only 6% of the annual total consumption of pefloxacin. Conversely, pefloxacin-resistant strains were detected in the medical cardiological wards to which the patients were usually transferred after surgery and had possibly been treated with pefloxacin. Resistant strains were very rarely isolated in the operating theatres in either building.

DISCUSSION

Pefloxacin, as other fluoroquinolones such as ciprofloxacin and ofloxacin, has several important microbiological and pharmacological advantages over other agents (7). They are bactericidal against a broad spectrum of aerobic bacteria, readily absorbed after oral administration, distributed in high concentrations to extravascular sites and have a low toxicity. In 1985, a French multicentre study determined the minimal inhibitory concentrations (MICs) of pefloxacin on 205 hospital CNS isolates to be between 0·06 and 8 μg/ml (MIC mode: 0·5) (5). In our hospital, pefloxacin has been frequently prescribed since 1985 particularly as an alternative to aminoglycosides. A total of 5·5 kg was used in 1988 for the treatment of Gram-negative and Gram-positive bacterial infections. Prophylaxis with pefloxacin was used during heart transplant surgery only.

CNS recovered from hospitalized patients displayed resistance to most commonly used anti-staphylococcal antibiotics (2). Oxacillin-resistance could be demonstrated in more than 60% of CNS recovered from routine clinical specimens (8). Allen (8) demonstrated that between 1975 and 1979, CNS resistance to methicillin, erythromycin and gentamicin had increased.

In our hospital, pefloxacin-resistance in clinical *S. epidermidis* increased in 2 years from 31 to 57%. Pefloxacin-resistant *S. epidermidis* isolates were also resistant to additional unrelated antibiotics, such as oxacillin, gentamicin, erythromycin, co-trimoxazole and fosfomycin. *S. epidermidis* pefloxacin resistance appeared to be predictive of the acquisition of hospital acquired multiple antibiotic resistance. The extension of the resistance patterns to include

gentamicin, erythromycin and trimethoprim could be due to the exchange of plasmids to CNS or to *S. aureus*, but no plasmid mediating resistance to quinolones has yet been described. The construction of the multiresistant phenotype of pefloxacin-resistant *S. epidermidis* could be understood by the analysis of the genetic basis of this resistance.

The selective pressure of hospital antibiotic use is implicated in the maintenance of the hospital reservoir of multiresistant CNS. Because of the broad spectrum activity of pefloxacin, resistant staphylococcal strains were selected in patients treated for infections caused by both Gram-positive and Gram-negative bacteria. Archer showed that antibiotic prophylaxis is an important factor in perpetuating a hospital reservoir for antibiotic-resistant CNS (9). The association between carriage rate and place of work is well recognized (9–13). In our study, staff and environmental colonization with pefloxacin-resistant *S. epidermidis* was rare when the antibiotic was used infrequently (operating theatres and medical neurological units). Conversely, colonization was found in 28–48% of the environmental samples and in 29–31% of the personnel working in units where the antibiotic was used frequently. Patients' bed-sheets in the linen room were also observed to be a vehicle of transmission of resistant strains.

From this work, we may conclude that the use of pefloxacin has selected for multiresistant strains of *S. epidermidis* which are spreading through the hospital environment. Whether this represents the dispersion of a resistant strain from a single source, or the acquisition of resistance by a number of different isolates can only be determined by the use of multiple epidemiological markers, particularly their plasmid profiles and endonuclease restriction patterns. The results of this investigation will be reported in a future communication.

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