

The epidemiology of ciprofloxacin resistance in coagulase-negative staphylococci in CAPD patients

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SUMMARY

Ciprofloxacin was used as empirical therapy for peritonitis in patients receiving continuous ambulatory peritoneal dialysis (CAPD) for 26 months, providing an opportunity to study the epidemiology of ciprofloxacin resistance amongst coagulase-negative staphylococci (CNS). Swabs were collected from the CAPD patients, staff, and clinic environment before, during and after the time this antibiotic was prescribed. Clinical isolates were also studied, and records kept of patient hospital attendance. Ciprofloxacin-resistant staphylococci were typed by antibiogram, biotype, plasmid profile, SDS-PAGE, and immunoblotting.

No resistant strains were detected before the use of ciprofloxacin. During its use 30% of patients became skin carriers, and resistant strains caused 8% of peritonitis episodes in 7% of patients (38% of carriers). Resistant strains were isolated from the environment, but never from attending members of staff. A total of 208 resistant isolates with MIC's between 8 and 128 mg/l was collected and identified as *Staphylococcus epidermidis* or *S. haemolyticus*. Sixteen strain types were distinguished. There was epidemiological evidence for selection of resistant strains derived from the host commensal flora and also for cross-colonization, and cross-infection. Carriage of resistant strains fell to 15% of patients, 6 months after the use of ciprofloxacin had ceased.

INTRODUCTION

Coagulase-negative staphylococci (CNS) are the most common cause of peritonitis in patients treated with continuous ambulatory peritoneal dialysis (CAPD) [1, 2]. We have previously demonstrated that certain infecting CNS can be detected amongst the commensal skin flora for up to 12 weeks before clinical infection, but not before this time [3]. The source of these infecting strains is not known. Possibilities include acquisition prior to infection perhaps by cross-infection in the clinic or in the home, or the presence of the organism in numbers too small to be detected by screening at an earlier time, or presence in an unscreened site.

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In November 1988 the fluoroquinolone, ciprofloxacin, was introduced as empirical therapy for CAPD peritonitis in our Renal Unit. This offered an opportunity to study the dynamics of resistance to ciprofloxacin amongst CNS in a self-contained population of patients. None of the quinolones, with the exception of nalidixic acid, had previously been available, and it was thus highly unlikely that either the patients or their commensal microbes had been exposed to the agent before the study began.

Antibiotic therapy as a selective agent for the evolution of resistance is widely accepted, but there have been few observations of the effect of antibiotic use on the colonizing and infecting strains of bacteria in a self-contained population of patients. Outbreaks of CNS have invariably occurred in association with prosthetic devices and on high-dependency units [4–6]. Two outbreaks of ciprofloxacin-resistant CNS have been reported in leukaemia units [7, 8], but in both studies screening was limited to short periods.

A study of the epidemiology of ciprofloxacin resistance might make it possible to answer some of the questions relating to the source of infecting strains. We wished to confirm that infecting strains colonize the skin first, and if this was so, determine their origin. Using conventional and molecular techniques to characterize the strains of CNS, we aimed to investigate the possibility of cross-colonization and cross-infection, and the possibility that strains might be able to adapt to selective pressure within their commensal environment on the host.

METHODS

Patients

All patients on the CAPD programme at St Thomas' Hospital were included in the study. Patients new to the programme were admitted to a renal ward prior to Tenckhoff catheter insertion, screened for *Staphylococcus aureus* carriage [9], and trained in aseptic care of the catheter exit wound by dedicated CAPD nurses [9, 10]. CAPD was carried out in the community, with a visit to the renal clinic every 6 weeks for a change of the Tenckhoff connector catheter. Records were kept of time and dates of clinic attendance and hospital admission.

Patients with peritonitis were seen by one of the CAPD nurses in a room on the renal ward. Peritonitis was diagnosed clinically and bacteriologically as previously described [11]. Ciprofloxacin was used as empirical therapy for CAPD peritonitis from November 1988 to January 1991 [12, 13]. Patients with peritonitis rarely had to be admitted, and most completed treatment out of hospital. Data of CAPD-associated peritonitis, including clinical presentation, microbiological findings, treatment and outcome were kept throughout the study.

Epidemiological survey

Skin swabs were collected over continuous 6-week periods in the out-patient clinic from the majority of the patients on the programme. Screening periods took place before ciprofloxacin therapy was introduced, after 9, 18 and 26 months of use, and finally 6 months after its use had ceased. Additional swabs were collected outside the formal screening periods from selected patients, including those new to the CAPD programme or known carriers of resistant strains.

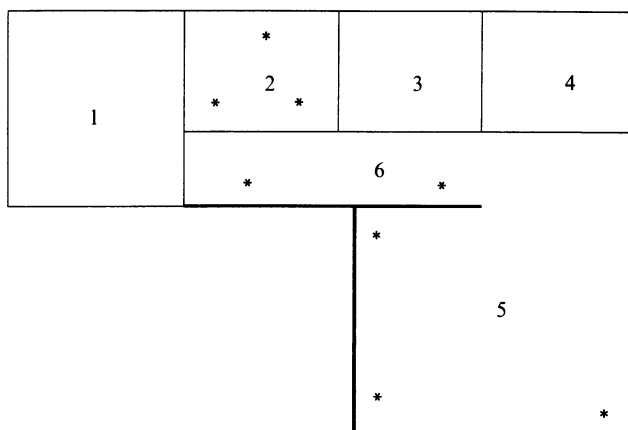


Fig. 1. Layout of the Renal Clinic. 1, examination room; 2, CAPD patient room; 3, transplant patient room; 4, reception; 5, waiting room; 6, corridor. *, Site of settle plates.

The following sites were sampled with cotton-wool tipped swabs moistened in sterile distilled water: nose, hands, axillae, umbilicus, perineum, Tenckhoff exit site and Tenckhoff tube titanium connector. Swabs were collected from the nose and hands of attending nursing staff at the beginning and at the end of clinics. Fomites in the CAPD patient room were sampled, including floor dust, patient chair, window sill, shelves, and the computer monitor screen. Swabs were stored in vials containing 0.5 ml glycerol 7% in Bacto Nutrient Broth (0003; Difco Laboratories) and frozen at -70°C [14]. Settle plates 14 cm in diameter and containing blood agar (7% horse blood in Columbia agar, Oxoid) were left for 4 h in the CAPD patient room, corridor and waiting room before aerobic incubation at 37°C for 24 h. The positions of the settle plates are illustrated in Fig. 1.

Microbiology

Screening swabs were thawed and cultured on blood agar and CLED (CM301, Oxoid) containing ciprofloxacin 4 mg/l to select resistant strains. After overnight incubation the number of CNS colonies was determined semi-quantitatively on both blood agar and the selective medium. Representatives of colonial types on the CLED selective agar were collected for further identification and typing. All epidemiological and clinical isolates were stored at -70°C in glycerol broth [14].

We have previously reported the evaluation of a typing scheme suitable for this epidemiological setting [15]. Isolates were initially typed by extended anti-biogram, biotype, and plasmid profile [16]. Phage typing, which we have previously shown to be poorly discriminatory, was not performed, but whole-cell protein typing by SDS-PAGE and immunoblotting was applied to all isolates [15].

Data storage and analysis

Patient, clinical and epidemiological data were stored and analysed on a microcomputer using dBASE III plus software (Ashton-Tate). The statistical significance of differences between carriage rates was assessed by the χ^2 test with Yates's correction [17].

Table 1. *Carriage of ciprofloxacin-resistant coagulase-negative staphylococci*

Total no. patients screened	97
Total no. of carriers	29
No. permanent carriers	24
No. transient carriers	5
Carriers who lost carriage at end of ciprofloxacin use	18
Carriers who retained carriage at end of ciprofloxacin use	11
Carriers infected by resistant CNS	11

Table 2. *Carriage rates of ciprofloxacin-resistant coagulase-negative staphylococci*

	Months of ciprofloxacin use in the renal unit				6 m after stopping
	0	9	18	26	
Number of:					
Patients screened	45	52	63	63	72
Patients carrying (%) resistant CNS	0 (0)	17 (33)	15 (24)	21 (33)	11 (15)
Patients with positive carriage and who had received ciprofloxacin	0	14	12	18	11
Patients with positive carriage and who had never received ciprofloxacin	0	3	3	3	0

RESULTS

Patients

Ciprofloxacin was used as empirical therapy for CAPD peritonitis for 26 months, during which time 166 patients with end-stage renal failure were managed on the CAPD programme at St Thomas' Hospital, and 105 of these received at least one course of ciprofloxacin during this period.

Skin carriage of ciprofloxacin-resistant CNS

It was not possible to swab all of the patients on the CAPD programme. Ninety-seven of 166 patients on the CAPD programme had a set of screening swabs collected before the use of ciprofloxacin (45 patients) or when they joined the programme during the study (52 patients), and subsequent sets of swabs collected during one or more of the screening periods during and after the use of ciprofloxacin on the unit. A total of 296 sets of specimens, comprising 2072 swabs, was collected.

No isolates of ciprofloxacin-resistant CNS were recovered from patients before the use of ciprofloxacin, or from patients joining the CAPD programme. Of the 97 patients screened throughout the study, 29 (30%) were found to be carriers (Table 1). The carriage rate of ciprofloxacin-resistant CNS after 9 months of ciprofloxacin use was 33%, after 18 months 24%, and at 26 months 33% (Table 2). Six months after the use of ciprofloxacin had ceased, the carriage rate had fallen to 15%, a significant reduction ($P = 0.012$, χ^2 test with Yates's correction). Forty-eight percent of carriers harboured more than one resistant strain (range 1-4 strains, mean 1.7). Fig. 2 illustrates the duration of carriage for each of the 29 carriers.

A total of 208 resistant CNS isolates was recovered from widely distributed sites

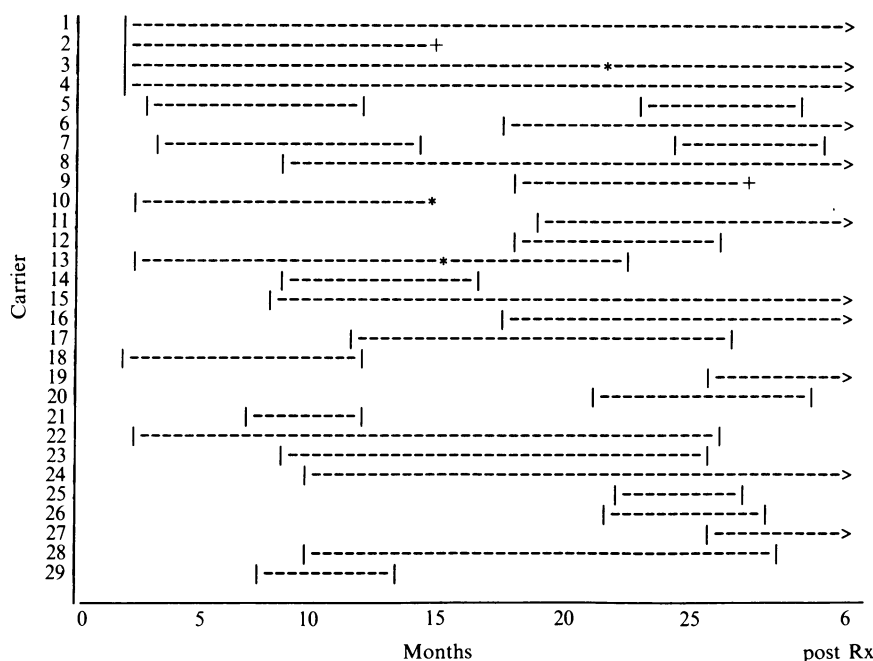


Fig. 2. Duration of carriage of ciprofloxacin-resistant CNS by 29 CAPD patients.
* Patient left the CAPD programme. + Patient died.

on the body: nose 36, hands 23, axilla 44, groin 55, umbilicus 39, Tenckhoff catheter exit site 4, catheter titanium connector 7. Resistant isolates were frequently the predominant organisms at these sites, but sensitive isolates were always present in addition. All resistant isolates were identified as *S. epidermidis* or *S. haemolyticus*, but several other staphylococcal species were represented amongst the sensitive isolates.

Resistant strains appeared to fall into two populations on the basis of their degree of resistance to ciprofloxacin. Resistant strains of *S. haemolyticus* and *S. epidermidis* had ciprofloxacin MIC's of 8–16 mg/l or 64–128 mg/l. Strain type 16 was *S. haemolyticus* with high-level resistance (128 mg/l). It was frequently isolated early in the study but was undetectable during the later screening periods. High-level resistance was observed most commonly amongst strains of *S. epidermidis*.

Typing

Typing of isolates revealed 16 distinguishable resistant strains whose characteristics are displayed in Table 3. The immunoblot analysis of a number of these strains are shown in Fig. 3. At 9 months 14 distinguishable strains were present (Table 4); at 18 months 14 strains, 2 of which were new (strain types 6 and 10), were isolated. Two uncommon strains (types 5 and 7), present at 9 months, were not recovered at 18 months. At 26 months only seven strains were recovered, and all of these had been recovered previously. At this time the majority of the isolates (53%) were indistinguishable by antibiogram plus biotype. Plasmid analysis and whole cell protein typing separated this predominant antibiotype into three distinct strains (types 4, 8, 9).

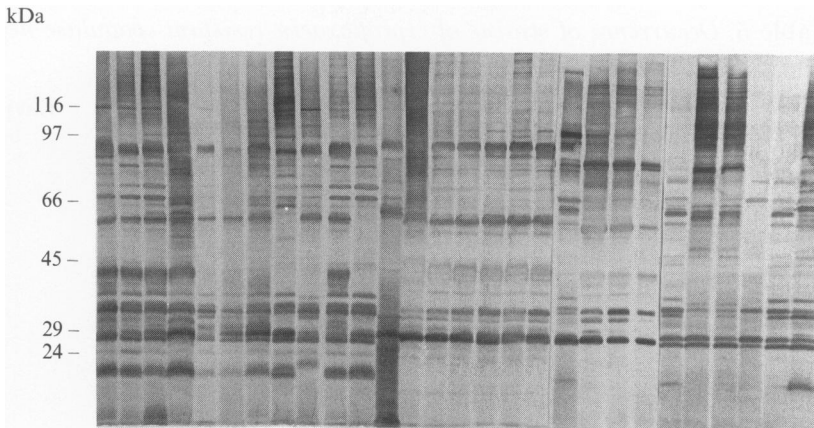
Table 3. Characteristics of 16 strains of ciprofloxacin-resistant coagulase-negative staphylococci

Strain type	Antibiogram†										Plasmid profile‡	SDS-PAGE‡	Immunoblot‡	MIC	Species							
	P	E	T	G	F	D	R	Y	V	C						U	S	N	O	X	M	
1*	P	E	T	G	F	D	R	Y	V	C	U	S	N	O	X	M	1	1	1	128	<i>Staphylococcus epidermidis</i>	
2*	P	E	-	G	-	D	-	Y	-	C	-	-	-	-	O	X	M	2	2	2	8	<i>Staphylococcus epidermidis</i>
3*	P	E	T	G	-	-	-	-	-	S	-	X	-	-	-	-	3	3	16	<i>Staphylococcus haemolyticus</i>		
4*	P	-	G	-	-	Y	-	-	-	-	O	X	M	-	-	-	4	4	128	<i>Staphylococcus epidermidis</i>		
5*	P	E	-	G	-	-	-	-	-	-	-	X	-	-	-	-	5	5	8	<i>Staphylococcus epidermidis</i>		
6*	P	E	-	-	-	-	-	-	-	-	-	O	X	M	-	-	6	1	10	<i>Staphylococcus epidermidis</i>		
7*	P	E	-	G	-	-	-	-	C	-	-	X	-	-	-	-	7	6	128	<i>Staphylococcus epidermidis</i>		
8	P	-	G	-	-	-	-	-	C	-	-	O	X	M	-	-	8	1	7	128	<i>Staphylococcus epidermidis</i>	
9	P	-	G	-	-	-	-	-	C	-	-	O	X	M	-	-	9	7	128	<i>Staphylococcus epidermidis</i>		
10	P	-	G	F	-	-	-	-	C	-	-	O	X	M	-	-	0	8	9	128	<i>Staphylococcus epidermidis</i>	
11	P	E	-	G	-	-	-	-	-	-	-	O	X	M	-	-	0	9	10	16	<i>Staphylococcus epidermidis</i>	
12	P	E	-	G	F	-	-	Y	-	-	S	-	O	X	M	-	10	3	11	128	<i>Staphylococcus haemolyticus</i>	
13	P	E	T	G	-	-	-	-	-	-	-	-	O	X	-	-	11	1	12	64	<i>Staphylococcus epidermidis</i>	
14	P	E	T	G	-	-	-	-	C	-	S	-	O	X	M	-	0	3	14	8	<i>Staphylococcus haemolyticus</i>	
15	P	-	-	-	-	-	-	Y	-	-	-	-	O	X	M	-	12	10	13	128	<i>Staphylococcus epidermidis</i>	
16	P	-	T	G	-	-	-	Y	-	C	-	S	-	O	X	M	13	3	14	128	<i>Staphylococcus haemolyticus</i>	

* Strains isolated from clinical infection.

† Capital letters in the antibiogram refer to resistance to the following antibiotics: P, penicillin; E, erythromycin; T, tetracycline; G, gentamicin; F, fusidic acid; D, clindamycin; R, rifampicin; Y, neomycin; V, vancomycin; C, chloramphenicol; U, mupirocin; S, streptomycin; N, novobiocin; O, trimethoprim; X, ciprofloxacin; M, methicillin; -, Sensitive.

‡ Numbers refer to distinct profiles for biotyping (ATB32 STAPH), plasmid typing, SDS-PAGE and immunoblotting; 0, not typable.



Strain type 1 1 1 1 2 2 4 5 8 9 7 os 3 3 3 3 3 3 11 16 16 14 6 10 10 12 13 15
 Immunoblot type 1 1 1 1 2 2 4 5 7 8 6 3 3 3 3 3 3 10 14 14 14 10 9 9 11 12 13

Fig. 3. Immunoblot of ciprofloxacin-resistant coagulase-negative staphylococci with their strain types and immunoblot profiles. OS, Control Oxford *S. aureus*.

Table 4. The isolation of strains of resistant coagulase-negative staphylococci from patients and environment during the use of ciprofloxacin in the Renal Unit

Strain	Before use	9 months	18 months	26 months	6 months after use
1	—	+ (22)	+ (20)	+ (25)	+ (32)
2	—	+ (5)	+ (3)	—	—
3	—	+ (13)	+ (14)	+ (12)	+ (16)
4	—	+ (5)	+ (12)	+ (23)	+ (25)
5	—	+ (1)	—	—	—
6	—	—	+ (2)	+ (4)	—
7	—	+ (1)	—	—	—
8	—	+ (11)	+ (12)	+ (14)	+ (10)
9	—	+ (11)	+ (14)	+ (16)	+ (17)
10	—	—	+ (2)	—	—
11	—	+ (1)	+ (1)	—	—
12	—	+ (5)	+ (7)	—	—
13	—	+ (1)	+ (1)	—	—
14	—	+ (5)	+ (2)	+ (6)	—
15	—	+ (1)	+ (1)	—	—
16	—	+ (17)	+ (9)	—	—

—, Not detected. +, Detected. (), % frequency of strain type.

Six months after the use of the antibiotic had ceased, five resistant strains could be detected. These strains had been recovered at every screening period since the introduction of ciprofloxacin. Two types (1 and 3) were the most frequently isolated from the environment, and three types (1, 3 and 4) had been responsible for 16 (73%) of 22 episodes of peritonitis caused by ciprofloxacin-resistant CNS.

Carriage of resistant CNS by staff and environmental contamination

Resistant CNS were never isolated from staff members (Table 5), but they were isolated from the environment on several occasions during each of the screening periods. Settle plates were the most productive means of recovery. Resistant CNS

Table 5. Occurrence of strains of ciprofloxacin-resistant coagulase-negative staphylococci

Strain type	Clinical isolate	Skin carriage	Staff isolate	Environment isolate
1	+ (6) 2	+ (45) 9	—	+ (4)
2	+ (3) 3	+ (9) 4	—	—
3	+ (8) 4	+ (39) 18	—	+ (4)
4	+ (2) 1	+ (19) 6	—	—
5	+ (1) 1	—	—	—
6	+ (1) 1	+ (7) 3	—	—
7	+ (1) 1	—	—	—
8	—	+ (26) 8	—	—
9	—	+ (22) 8	—	+ (3)
10	—	+ (2) 1	—	—
11	—	+ (3) 2	—	—
12	—	+ (6) 2	—	—
13	—	+ (2) 1	—	—
14	—	+ (7) 2	—	—
15	—	+ (2) 1	—	—
16	—	+ (21) 6	—	+ (3)

(x), number of isolates, the number outside parentheses refers to the number of patients affected.

were almost exclusively isolated from settle plates in the CAPD patient room (Fig. 1), only one colony being recovered from the waiting room, and none from the corridor. Only 4 of 36 fomite swabs yielded resistant isolates, 3 from the patient chair and 1 from the floor in the CAPD patient room. Seven of 14 environmental isolates were identified as *S. haemolyticus*, and seven as *S. epidermidis*. These isolates represented four distinct strains. Two strains, types 9 and 16, were indistinguishable from skin strains recovered from patients swabbed in the clinic on the same day, and two strains (types 1 and 3) were widespread skin colonizers and were indistinguishable from strains causing clinical infection (Tables 4, 5).

Clinical infections

During the study period, 284 episodes of CAPD peritonitis in 105 patients were treated with ciprofloxacin. Twenty-two (8%) episodes in 11 (7%) patients were caused by coagulase-negative staphylococci resistant to ciprofloxacin (MIC > 4 mg/l). All 11 patients were carriers of resistant CNS before infection. Thus 11 of 29 carriers (38%) became infected with resistant strains. The occurrence of these episodes in relation to the number of months that ciprofloxacin had been in use is illustrated in Fig. 4.

Typing of the 22 clinical isolates by antibiogram plus biotype revealed seven resistant strains (Table 3). Minor differences in antibiogram and/or biotype were regarded as insignificant if the supplementary typing methods showed the isolates to be otherwise indistinguishable. Three strains were responsible for peritonitis in more than one patient. Strain type 1, identified as *S. epidermidis*, caused six episodes in two patients. Both patients attended the clinic on the same day, and only the first patient was known to be a carrier of ciprofloxacin-resistant CNS at the time. Strain type 2, *S. epidermidis*, caused one episode in each of three

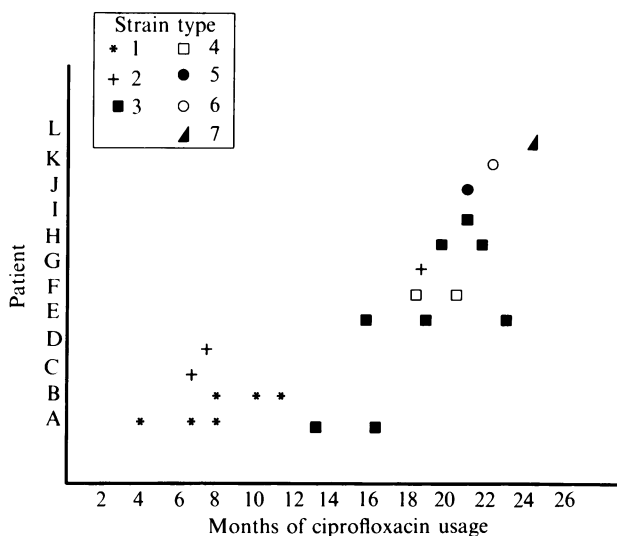


Fig. 4. The occurrence of peritonitis caused by ciprofloxacin-resistant coagulase-negative staphylococci.

Table 6. Evidence for de novo emergence of ciprofloxacin resistance

Patient	Data sensitive strain isolated	MIC mg/l	Date of ciprofloxacin RX	Date resistant strain isolated	MIC mg/l	Species
1 (LA)	11 Oct. 1988	0.5	3 Jul. 1989	13 Nov. 1989	8.0	<i>S. haem</i>
2 (LC)	3 May 1988	0.25	17 Mar. 1989	19 Oct. 1989	128	<i>S. epid</i>
3 (AM)	15 Oct. 1989	2.0	25 Apr. 1990	16 Oct. 1990	16.0	<i>S. haem</i>
4 (RJ)	15 Feb. 1989	0.25	3 Jul. 1989	19 Nov. 1989	128	<i>S. epid</i>

patients. The first two of these patients attended clinic on the same day and 2 weeks later were in adjacent beds on the ward. Both their episodes of peritonitis occurred within a week of each other, 1 month later. The following year the third patient who was not a carrier was in the ward for Tenckhoff catheter insertion at the same time as the second patient, who continued to carry strain type 2. These patients also shared attendance at the clinic on two occasions, before the third patient developed peritonitis caused by strain type 2.

Strain type 3, *S. haemolyticus*, caused eight episodes in four patients. Dates of clinic attendance coincided for three of these four patients and this strain was also isolated from settle plates in the clinic on a day when one of these patients attended.

Evidence for de novo development of resistance

In four patients early isolates of skin CNS sensitive to ciprofloxacin had antibiotypes, identical apart from ciprofloxacin sensitivity, to resistant organisms isolated at a later date (Table 6). Supplementary typing methods confirmed that these sensitive and resistant strains were otherwise indistinguishable (Fig. 5). The sensitive isolates were recovered before the patient was exposed to ciprofloxacin and the resistant isolates after first exposure to the agent.

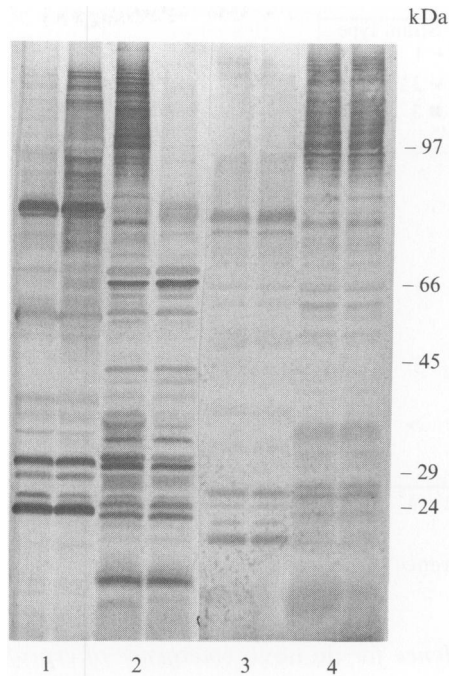


Fig. 5. Immunoblot of coagulase-negative staphylococci demonstrating *de novo* emergence of ciprofloxacin resistance. Each pair of tracks is numbered, and these refer to the numbers in Table 6. For each of the four pairs of isolates the left-hand track is the sensitive organism and the right track the resistant one.

Table 7. Evidence for cross-colonization. Patients who became carriers of resistant strains but who had not been treated with ciprofloxacin

Patient	Strain type	MIC mg/l	Probable patient source	Date of contact
1 (IS)	16	128	(CM)	5 Oct. 1989
2 (SL)	3	32	(DC)	9 Oct. 1989
3 (IJ)	3	8	(AM)	24 Jun. 1990*
4 (CC)	1	128	(BC)	5 Jan. 1990
5 (FP)	2	8	(JH)	4 Jun. 1990
6 (MS)	9	128	(CM)	5 Oct. 1989
7 (JH)	8	128	Not known	After 23 May 1990
8 (LK)	4	64	Not known	After 9 Feb. 1990
9 (RP)	12	128	Not known	After 30 Apr. 1990

* Strain 3 also isolated from clinic environment on this date.

Evidence for cross-colonization and cross-infection

During the study period nine patients who had not been shown to carry resistant strains, and who had never received ciprofloxacin, were subsequently found to be carriers of resistant strains (Table 7). Following Tenckhoff catheter insertion, one patient had shared a ward bay with another CAPD patient known to be a carrier. Unfortunately, neither patient was screened while on the ward, but at subsequent out-patient clinics both patients were found to be carrying indistinguishable strains of *S. haemolyticus*.

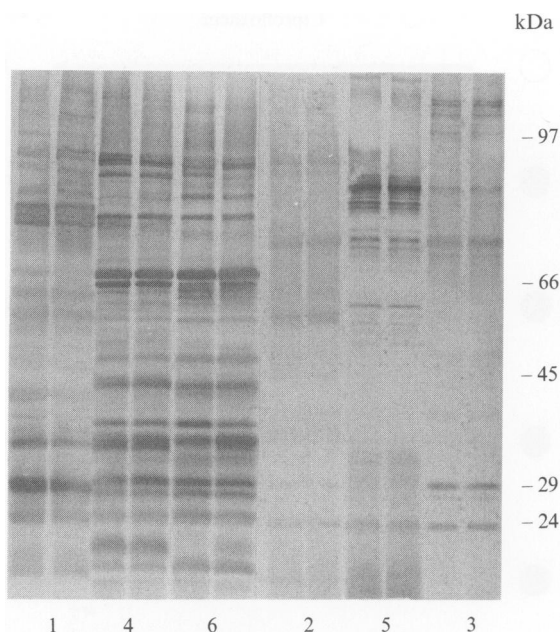


Fig. 6. Immunoblot of isolates of coagulase-negative staphylococci demonstrating cross-colonization. Each pair of tracks is numbered and these refer to the numbers in Table 7. For each pair of tracks, the left is the isolate from the donor patient, the right from the probable source patient. Immunoblot of strains demonstrating *de novo* selection of resistance.

In six of the nine patients who acquired a resistant strain it was possible to correlate a clinic visit with that of a known carrier of the resistant strain. On one such occasion the resistant strain was also isolated from the clinic environment. Fig. 6 shows the immunoblot of isolates from the suspected donor patients and from the recipients. None of these nine patients developed clinical infection with their resistant strains.

Eight of the most widespread strain types were implicated in cross-colonization. One patient (CM) appeared to have been responsible for the spread of two different strains to two different patients on the same day.

DISCUSSION

Although CNS are increasingly important as hospital pathogens, their epidemiology is poorly understood. One reason for this has been the difficulty in strain characterization. It was only relatively recently that the more accurate identification of CNS species has been possible in the routine laboratory [19, 20]. Characterization of CNS has been further fraught with problems of typability, discrimination and reproducibility. Recent studies have used a variety of techniques to distinguish strains: antibiogram, biotype, phage susceptibility and plasmid profile [16] and recently molecular techniques [21]. We evaluated whole cell protein typing by SDS-PAGE and immunoblotting [15] and applied it to this study.

It is assumed that CNS strains causing peritonitis are derived from the patient's

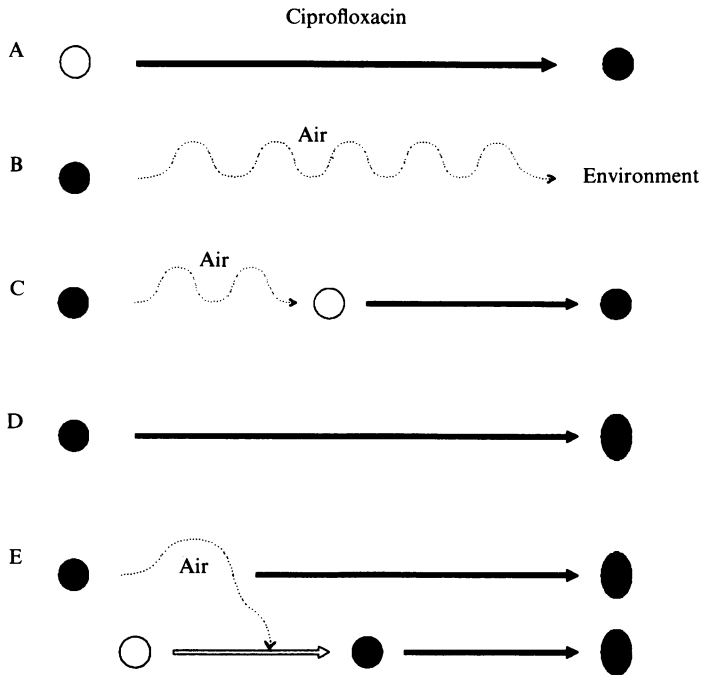


Fig. 7. Diagrammatic representation of the epidemiology of ciprofloxacin-resistant coagulase-negative staphylococci. A, Non-carriers may become carriers after therapy with ciprofloxacin. B, Carriers may contaminate the environment with resistant strains via air transmission. C, Cross-colonization via air transmission may occur from a carrier to a non-carrier. D, Carriers may become infected with resistant strains. E, Cross-infection may occur. ○, Non-carrier; ●, carrier; ◐, infected patient.

own skin flora, although a number of studies have been unsuccessful in demonstrating this [11, 23]. Ludlam and co-workers however, successfully demonstrated that infecting strains are present as commensal flora in CAPD patients for up to 12 weeks before the patient exhibits clinical infection, but not before this [3]. In studying the dynamics of ciprofloxacin-resistant CNS in a population of CAPD patients before, during and after exposure to the agent, answers might be provided to some of the remaining questions relating to the epidemiology of these organisms in general.

Our view of the epidemiology of ciprofloxacin resistant CNS is summarized in Fig. 7. Ciprofloxacin resistance was not detected among commensal CNS before this antibiotic was used in the Unit, or in patients who were new to the CAPD programme. Resistant isolates emerged only after exposure of CAPD patients to ciprofloxacin (Fig. 7A). Carriage of resistant strains could be demonstrated in approximately one third of patients and this rate remained relatively constant while the antibiotic was in use, falling to 15% 6 months after the agent was discontinued.

The great majority of carriers (83%) retained their resistant CNS throughout the use of ciprofloxacin in the Unit. A small proportion (17%) were transient carriers, in most cases, of strains such as 5, 7, 10 and 15 which were uncommon. Once the selective pressure of ciprofloxacin had been removed, a number of permanent carriers lost their resistant strains, but 38% of carriers still retained

resistant strains 6 months later. Multiple resistance to antibiotics other than ciprofloxacin is common among CNS even in untreated patients [24, 25]. However, in the case of a recently introduced antibiotic such as ciprofloxacin, resistance would appear to emerge only after exposure to the agent. Others have observed the association between antibiotic use and increased resistance [25].

Spread of resistant strains occurred, and transmission was demonstrated to be via an air-borne route in the out-patient clinic (Fig. 7 B). Resistant strains were isolated from the environment, most commonly from settle plates, and patients in the clinic. Cross-colonization was also observed (Fig. 7 C) in six non-carriers who had never received ciprofloxacin and who acquired carriage after contact with known carriers in the clinic. Cross-colonization was probably more widespread, because carriage of certain common strains was detected in many patients. The nursing staff were never implicated in transmission, and were never demonstrated to be carriers, which may be explained by their rigorous aseptic procedures [10]. That cross-colonization of antibiotic-resistant CNS strains may occur between CAPD patients has been suspected [26], but never previously proved.

The results of this study differ from other reports of outbreaks of ciprofloxacin-resistant CNS. Two outbreaks [7, 8] were characterized by the spread of single strains of ciprofloxacin-resistant *S. epidermidis* within a population of leukaemic patients treated with this antibiotic. Oppenheim and colleagues observed a single, highly resistant, strain (MIC \geq 128 mg/l) which caused clinical infection, and patient and environmental colonization, but not staff colonization [7]. Transmission of the resistant strain was believed to be via an air-borne route. In the second study [8], Kotilainen and co-workers detected carriage of the resistant strain by members of the staff and implicated them in transmission. As the resistant isolates were reported to have varying MICs of ciprofloxacin, and similar but not indistinguishable plasmid profiles, it is questionable whether a single outbreak strain was present in this study. They concluded that the outbreak strain had been transmitted by direct contact between patients and staff. In a further report, a number of CNS strains resistant to ciprofloxacin and/or gentamicin were found to be widespread in a bone-marrow transplant unit [27], and it was speculated that transmission of resistant strains may be by air or by passive transfer between staff and patients. Quinolone-resistant CNS were found to be more widespread in hospital units in which the quinolone perfloracin was used most frequently [28]. None of these studies was able to show that resistance to the quinolone emerged only after use of the antibiotic in the units.

Acquisition of resistant strains from external sources is believed to be more common than development of resistance by endogenous flora [25]. However, in four CAPD patients it was possible to demonstrate that development of resistance by endogenous strains occurred *de novo* following exposure to the antibiotic. In these patients, sensitive strains of CNS isolated before exposure to ciprofloxacin were indistinguishable by SDS-PAGE and immunoblot from ciprofloxacin-resistant strains isolated after exposure.

The first clinical infection caused by a resistant strain occurred 4 months after introduction of the agent. Seven per cent of CAPD patients, but 38% of carriers, became infected with resistant strains, in most cases by strains previously isolated from their skin (Fig. 7 D). This observation adds further weight to the concept that

CNS peritonitis is caused by strains which have previously colonized the skin [3]. Over the next 22 months, episodes of infection with seven distinguishable, resistant strains were largely sporadic. There were compelling evidence for cross-infection (Fig. 7E). One strain caused six episodes in two patients, and transmission probably occurred in the CAPD clinic. A second strain caused three episodes in three patients. Transmission in these patients may have occurred either in the CAPD clinic or in the ward. A third strain caused eight episodes in four patients, and in this case transmission almost certainly occurred in the CAPD clinic, as the strain was also isolated from settle plates on the relevant day.

Supplementary typing methods were essential for this investigation. Antibigram plus biotype was not sufficiently discriminatory, for example in distinguishing between resistant strains 4, 8 and 9 (Table 3). However in other instances the antibiogram was too discriminatory. Clinical isolates were falsely distinguished by antibiogram, but were clearly indistinguishable by other typing methods. Plasmid typing successfully characterized the majority of strains, but a number of isolates could not be typed by this method. Whole-cell protein typing was labour intensive, but typed all the isolates.

The observations of the frequency of carriage of resistant strains requires further comment. For the first 18 months of ciprofloxacin use there was a wide diversity of resistant strains (14 strains were detected at each screening period) (Table 4). Six strains (1, 3, 4, 8, 9 and 16), colonizing many patients, were most commonly isolated. Uncommon strains such as 5, 7 and 10 were only detected during one screening period and they colonized few patients. At 26 months, the diversity of strains was much narrower with only seven strains being detected. Most of the more common strains (1, 3, 4, 8 and 9) persisted, but strain 16 which had been the second most widespread strain at 8 months had disappeared. At 26 months no new strains were detected. It would seem that use of ciprofloxacin rapidly selected a wide variety of resistant strains, and that some of these were more successful than others in their ability to persist and disseminate.

Only 7 of 16 resistant strains caused clinical infection, but these 7 strains were not necessarily the most common. Strains 2, 5, 6 and 7 represented 27% of the clinically significant isolates but only 7.6% of the skin isolates. On the other hand strain 16, commonly isolated in the early part of the study, never caused a clinical infection. These observations argue for possible pathogenicity for certain strains in the peritoneal cavity and this would be worthy of further investigation.

The range of MICs of ciprofloxacin (8–128 mg/l) in resistant strains suggested that more than one mechanism of resistance was occurring. The only known genetic mechanism of resistance to the fluoroquinolones is chromosomal mutation, often affecting the gene coding for DNA gyrase [29]. Permeability mechanisms might play a role and these might explain the wide variation in the level of resistance. In this regard we are investigating the outer membrane proteins of resistant strains. It is possible that the genetic information coding for permeability changes could be carried on plasmids. This hypothesis was tested by performing plasmid transfer between resistant CNS and a recipient strain of *S. aureus*. Although resistance to gentamicin, tetracycline and chloramphenicol could be transferred, it was not possible to transfer ciprofloxacin resistance. Further investigation of the chromosomal mechanisms of resistance in these strains is

indicated. This could be most effectively achieved by amplifying the *gyrA* gene by the polymerase chain reaction, and determining its nucleotide sequence, a technique which we are undertaking at present.

Following withdrawal of the antibiotic from the Unit for economic rather than therapeutic reasons, there were no further clinical infections with ciprofloxacin-resistant CNS. Although ciprofloxacin-resistant strains had caused 7% of clinical infections, this resistance rate was not sufficiently high to prejudice the use of ciprofloxacin as empirical therapy which retained an overall cure rate of about 80% [12, 13].

Although it is impossible to tell whether ciprofloxacin-resistant strains are a model for all CNS, it seems unlikely that further infection control measures would reduce transmission and the rate of CNS peritonitis. The organisms, resistant or otherwise, are ubiquitous as commensals. Eradication of the source is impossible and, with our current strict procedures for aseptic care of the Tenckhoff catheter site, transmission from the skin of the patient to the Tenckhoff catheter is probably reduced to a minimum. The incidence of CNS peritonitis can be further reduced by the flushing mechanism of the Y-set Tenckhoff connector [30–32].

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REFERENCES

1. Ludlam HA, Dryden MS, Wing AJ, Phillips I. The prevention of peritonitis in continuous ambulatory peritoneal dialysis. *Lancet* 1990; **335**: 1161.
2. Bint AJ, Finch RG, Gokal R, Goldsmith HJ, Junor B, Oliver D. Diagnosis and management of peritonitis in continuous ambulatory peritoneal dialysis. (Report of a Working Party of the British Society for Antimicrobial Chemotherapy). *Lancet* 1987; *i*: 845–9.
3. Ludlam HA, Noble WC, Marples RR, Bayston R, Phillips I. The epidemiology of peritonitis caused by coagulase-negative staphylococci in continuous ambulatory peritoneal dialysis. *J Med Microbiol* 1989; **30**: 167–74.
4. Marples, RR. The role of typing of coagulase-negative staphylococci in hospital-acquired infection. *J Hosp Infect* 1984; **5** Suppl. A: 51–5.
5. Houang ET, Marples RR, Weir I, Mourant AJ, de Saxe M, Singleton B. Problems in the investigation of an apparent outbreak of coagulase-negative staphylococcal septicaemia following cardiac surgery. *J Hosp Infect* 1986; **8**: 224–32.
6. Dandalides PC, Rutala WA, Tomann CA, Sarubbi FA. Serious postoperative infections caused by coagulase-negative staphylococci: an epidemiological and clinical study. *J Hosp Infect* 1986; **8**: 233–41.
7. Oppenheim BA, Hartley JW, Lee W, Burnie JP. Outbreak of coagulase-negative staphylococcus highly resistant to ciprofloxacin in a leukaemia unit. *Br Med J* 1989; **229**: 294–7.
8. Kotilainen P, Nikoskelainen J, Huovinen P. Emergence of ciprofloxacin-resistant coagulase-negative staphylococcal skin flora in immunocompromised patients receiving ciprofloxacin. *J Infect Dis* 1990; **161**: 41–4.
9. Ludlam HA, Young AE, Berry AJ, Phillips I. The prevention of infection with *Staphylococcus aureus* in continuous ambulatory peritoneal dialysis. *J Hosp Infect* 1989; **14**: 293–301.

10. Dryden MS, Ludlam HA, Wing AJ, Phillips I. Active intervention dramatically reduces CAPD-associated infection. *Adv Peritoneal Dialysis* 1991; **7**: 125-8.
11. Ludlam HA, Price TCN, Berry AJ, Phillips I. Laboratory diagnosis of peritonitis in patients on continuous ambulatory peritoneal dialysis. *J Clin Microbiol* 1988; **26**: 1757-62.
12. Ludlam HA, Barton I, White L, McMullin C, King A, Phillips I. Intraperitoneal ciprofloxacin for the treatment of peritonitis in patients receiving continuous ambulatory peritoneal dialysis. *J Antimicrob Chemother* 1990; **25**: 843-51.
13. Dryden MS, Wing AJ, Phillips I. Low-dose intraperitoneal ciprofloxacin for the treatment of peritonitis in patients receiving continuous ambulatory peritoneal dialysis. *J Antimicrob Chemother* 1991; **28**: 131-9.
14. Ludlam HA, Nwachukwu B, Noble WC, Swan AV, Phillips I. The preservation of micro-organisms in biological specimens stored at -70° . *J App Bacteriol* 1989; **67**: 417-23.
15. Dryden MA, Talsania H, Martin S, et al. Evaluation of supplementary methods for typing coagulase-negative staphylococci. *J Med Microbiol* 1992. In press.
16. Ludlam HA, Noble WC, Marples RR, Phillips I. The evaluation of a typing scheme for coagulase-negative staphylococci suitable for epidemiological studies. *J Med Microbiol* 1989; **30**: 161-5.
17. Swinscow TD. *Statistics at square one*, 2nd edn. London: British Medical Association, 1976: 43-53.
18. Phillips I. *A guide to sensitivity testing*. Report of the Working Party on Antibiotic Sensitivity Testing of the British Society for Antimicrobial Chemotherapy. London: Academic Press, 1991.
19. Kloos WE, Schleifer KH. Isolation and characterisation of staphylococci from human skin. II. Description of four new species: *S. warneri*, *S. capitis*, *S. hominis* and *S. simulans*. *Internat J System Bacteriol* 1975; **25**: 62-79.
20. Kloos WE, Schleifer KH. Simplified scheme for routine identification of human *Staphylococcus* species. *J Clin Microbiol* 1975; **1**: 82-8.
21. Burnie JP, Lee W, Matthews RC, Bayston R. Immunoblot fingerprinting of coagulase negative staphylococci. *J Clin Pathol* 1988; **41**: 103-7.
22. Eisenberg ES, Ambalu MS, Szylagi G, Aning V, Soeiro R. Colonisation of skin and development of peritonitis due to coagulase-negative staphylococci in patients undergoing peritoneal dialysis. *J Infect Dis* 1987; **156**: 478-82.
23. Beard-Pegler MA, Gabelish CL, Stubbs E, et al. Prevalence of peritonitis-associated coagulase negative staphylococci on the skin of continuous ambulatory peritoneal dialysis patients. *Epidem Infect* 1989; **102**: 365-78.
24. Cove JH, Eady EA, Cunliffe WJ. Skin carriage of antibiotic-resistant coagulase-negative staphylococci in untreated subjects. *J Antimicrob Chemother* 1990; **25**: 459-69.
25. Thore M, Kuhn I, Lofdahl S, Burman LG. Drug-resistant coagulase-negative staphylococci. *Epidemiol Infect* 1990; **105**: 95-105.
26. Degener JE, Naidoo JL, Noble WC, Phillips I, Marples RR. Carriage of gentamicin-resistant coagulase-negative staphylococci in patients on continuous ambulatory peritoneal dialysis. *J Antimicrob Chemother* 1987; **19**: 505-12.
27. Hedin G, Hambræus A. Multiply antibiotic-resistant *Staphylococcus epidermidis* in patients, staff and environment - a one-week survey in a bone-marrow transplant unit. *J Hosp Infect* 1991; **17**: 95-106.
28. Etienne J, Brun Y, Billard M, Fleurette J. Hospital dispersion of *Staphylococcus epidermidis* isolates resistant to a fluoroquinolone, pefloxacin. *Epidemiol Infect* 1989; **103**: 459-64.
29. Neu HC. Bacterial resistance to fluoroquinolones. *Rev Infect Dis* 1988; **10**: 577-63.
30. Verger C, Luzar MA. In vitro study of CAPD Y line systems. In: Khanna R, et al. eds. *Advances in continuous ambulatory peritoneal dialysis*. Peritoneal Dialysis Bulletin Inc 1986: 160-4.
31. Maiorca R, Cancarini GC, Broccoli R, et al. Prospective controlled trial of a Y-connector and disinfectant to prevent peritonitis in continuous ambulatory peritoneal dialysis. *Lancet* 1983; **ii**: 642-4.
32. Dryden MS, McCann M, Wing AJ, Phillips I. Controlled trial of a Y-set delivery system to prevent peritonitis in patients receiving continuous ambulatory peritoneal dialysis. *J Hosp Infect* 1992; **20**: 185-92.