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Article

Epidemiology of Bacterial Infection During Management of Open Leg Fractures

H. Carsenti-Etesse, F. Doyon, N. Desplaces, O. Gagey, C. Tancrède, C. Pradier, B. Dunais, P. Dellamonica, and the Study Group

Abstract In a randomised double-blind trial conducted between 1990 and 1994, 616 patients from 43 centres, pefloxacin (group P, 316 patients) and a cefazolin-oxacillin combination (group C, 300 patients) were compared in the prophylaxis of bone infection after grade 1 and 2 open leg fractures. Samples were obtained at emergency, before and during surgery, and from drain aspirates. Antimicrobial susceptibility, slime production and adherence properties of the bacteria were tested. Cultures at emergency and before surgery showed similar distributions of gram-positive and gram-negative bacteria in both groups, while wound closure and infecting isolates showed prevailing gram-positive bacteria in group P and gram-negative bacteria in group C. Positive cultures at each stage were correlated with the occurrence of infection but were not predictive of the infecting species, which were nosocomial bacteria in most cases. Positive cultures at wound closure warn of a higher infection risk. Twenty-one of 316 (6.6%) patients in group P and 24 of 300 (8%) in group C were considered infected within 3 months. The difference is not significant (chi-square test $=0.42$; $P=0.51$). Infecting strains were isolated from 38 patients (group P, 18; group C, 20). Infecting species,although not predictable, appear to be those escaping the spectrum of the prescribed antimicrobial prophylaxis.

Introduction

Infection remains the primary cause of nonunion and bone instability in open fractures, which are graded according to the degree of soft tissue injury associated with the fracture. Reported rates of infection range from 0 to 9% for grade 1 fractures, from 1 to 12% for grade 2 fractures, and from 9 to 55% for grade 3 fractures. Grade 1 fractures are those with a skin wound less than 1 cm long and without significant soft tissue damage. The skin wound is usually caused by puncture of a bone fragment from within. Grade 2 fractures have larger wounds or underlying tissue damage. The skin wounds are usually caused by laceration by an external object. Grade 3 fractures include open segmental fractures; those with extensive soft tissue damage, crushing, flaps, or avulsions; and those with accompanying neurologic or vascular injuries requiring repair [1].

Obtaining preoperative cultures from open fracture wounds has long been considered part of open fracture management protocols, their results being considered predictive of bacterial involvement in infection [2]. Prophylactic antibiotics can reduce the rate of infection following orthopaedic procedures. The purpose of this study was to assess the correlation between organisms grown from pre- and peri-operative cultures and those grown from infected sites, as well as to determine

H. Carsenti-Etesse (✉), C. Pradier, B. Dunais, P. Dellamonica
Service des Maladies Infectieuses et Tropicales, Hôpital de l'Archet I, Route de St. Antoine de Ginestière, BP 79, F-06202 Nice Cedex, France

F. Doyon
INSERM U 287, Institut Gustave Roussy, F-94805 Villejuif Cedex, France

N. Desplaces
Laboratoire de Microbiologie, Hôpital de la Croix Saint Simon, 125 Rue d'Avron, F-75960 Paris, France

O. Gagey
Hôpital de Bicêtre, 78 Rue Général Leclerc, F-94276 Le Kremlin Bicêtre, France

C. Tancrède
Laboratoire de Microbiologie, Institut Gustave Roussy, F-94805 Villejuif Cedex, France

whether the proven effectiveness of pefloxacin in bone infection might be extended to prophylaxis [3, 4].

Patients and Methods

This double-blind, placebo-controlled, multicentre randomised study included 616 patients from 43 centres after informed consent was obtained. It was supervised by a data monitoring committee.

Eligibility Criteria. Eligible patients were adults over 15 years of age with an open extra-articular fracture of the tibia requiring reduction fixation and wound closure that could be performed directly or with a muscle or skin plasty (grades 1 and 2). The delay between fracture and surgery was not to exceed 12 h.

Study Medications. Patients ware randomly assigned to receive either a single 800 mg dose of pefloxacin i.v. (group P) or a 2-day course of cefazolin i.v. (1 g q.i.d.; total dose 8 g) followed by a 3-day course of oral oxacillin (1 g t.i.d.) (group C). The antimicrobial prophylaxis was given with anaesthetic induction.

The protocol included prophylaxis against anaerobic infections by penicillin G (20×10^6U/day for 2 days) or metronidazole/ornidazole according to the current protocol applied by the physicians in each specific centre. Other antibiotics were prohibited during the 5 days after surgery.

Follow-up. Patients were recalled 3 months after their operation.

Endpoints. The primary endpoint was a wound infection occurring within 3 months: this was defined as a fistula, a local inflammatory aspect with isolation of a pathogenic organism, reoperation within 3 months for suspected infection, or isolation of pathogenic organisms from the surgical site when the patient was reoperated for another reason.

Bacteriological Investigations. Four samples were collected systematically by swabbing a 1 cm^2 surface over the fracture site or within its immediate vicinity at various stages: in the emergency ward, at the beginning of surgery prior to application of antiseptics, and the end of surgery prior to wound closure. Swabs were placed in a transport medium (Portagerm; bioMérieux, France). Needle aspirates of the drain tube at day 2 after surgery were cultured in broth (Hémoline; bioMérieux). If infection was suspected, one of the following procedures was followed: (i) needle aspiration of the fracture site or drain tube; (ii) in the presence of a fistula, deep insertion of a swab after skin disinfection; or (iii) bone biopsy and soft tissue sample in the event of a second surgical procedure.

Laboratory Methods. Identification of bacteria and determination of antimicrobial susceptibility were performed by standard bacteriological procedures (susceptibility determined by the disk diffusion test) in each site. All strains isolated were sent to a central laboratory for determination of MICs of pefloxacin, cefazolin, and oxacillin by the macrobroth dilution method according to National Committee for Clinical Laboratory Standards procedures. Slime and adherence properties of most strains were also determined by the procedures described by Christensen et al. [5].

Sample Size. In order to detect a difference between infection rates of 10% in group C and 5% in group P with a type I error of 5% and a power of 80%, 500 patients were calculated to be required in each treatment group, thus a total of 1000 patients.

Randomisation. Boxes containing either (a) pefloxacin and the cefazolin-oxacillin placebo or (b) cefazolin-oxacillin and the pefloxacin placebo were numbered according to a random list that was balanced every four numbers. In each centre, just before operation, patients had to receive the lowest numbered box available. The code was made available to the attending physician in a sealed envelope in each box.

The data monitoring committee included the coordinating members of the trial and three independent members: a bacteriologist, an orthopaedic surgeon, and an infectiologist. The members made a blind review of all reported infections and of all cases reporting reoperation within 3 months after randomisation.

Statistical Analysis. Data analysis was performed on an intent-to-treat basis on all randomised patients. Qualitative variables were compared using the chi-square-test or Fisher's exact test, and quantitative variables were compared using Student's *t*-test.

Results

A total of 616 patients were included in the trial, 316 in group P and 300 in group C. Among these, 21 (6.6%) patients included in group P and 24 (8%) in group C were infected within 3 months. The difference is not significant ($P = 0.51$). Samples obtained from 38 infected patients were found positive. There was no significant difference in bacterial contamination between the two treatment groups at any sampling stage ($P > 0.05$) (Table 1). Local irrigation with antiseptics had been performed during transport for 17% and 20% of patients in each group, and for 37% of patients in each group at emergency.

Bacterial Epidemiology. In both treatment groups, bacterial contamination at the fracture site consisted of a comparable distribution of gram-negative (25%) and gram-positive (75%) species upon arrival at the emergency department and at the start of the operation (Table 2). Among gram-positive species, staphylococci accounted for 70% of the species cultured, with methicillin-sensitive coagulase-negative staphylococci (MSCNS) isolated most often.

Staphylococcus aureus accounted for 8% of staphylococci and was always methicillin-sensitive *Staphylococcus aureus* (MSSA). Among gram-negative bacteria, *Acinetobacter* spp. and *Enterobacter* spp. were similarly represented in both treatment groups and together accounted for 60% of gram-negative bacteria.

There was a difference between the proportions of gram-positive and gram-negative bacteria isolated at wound closure ($P < 0.01$): gram-positive bacteria accounted for 35 of 37 (94.6%) species isolated in group P and for 19 of 32 (59.4%) in group C. In group P bacteria mainly consisted of staphylococci (20/37 isolates, 57%), essentially coagulase-negative staphylococci (1 methicillin-resistant isolate). The proportion of *Staphylococcus aureus* was comparable to that found at prior stages (3/20 isolates), and the isolates remained susceptible to methicillin. In group C *Staphylococcus*

Table 1 Contamination rates at fracture sites at each stage of sampling and in each treatment category

Sampling stage	No. of patients sampled	Contamination rate (%)			*P* value
		All patients	Pefloxacin group	Cefazolin + oxacillin group	
Presentation to ED	570	28.0	26.3	30.0	0.33
Start of surgery	587	16.3	15.4	17.3	0.54
Wound closure	583	9.9	9.8	9.0	0.79
Drain aspirate	531	6.0	6.1	6.0	0.95
Infection	38	6.3	5.8	6.7	0.52

ED, emergency department

spp. represented ten of 19 (52.6%) strains. MSCNS were most often present (80%); no strain was methicillin resistant. There were two isolates of MSSA.

Table 3 lists the isolates cultured from each patient at wound closure. At wound closure, 27 new strains were cultured from 17 patients colonised with different species and from six patients with previously sterile samples. Thirty-five patients were found to carry strains already isolated at prior sampling stages. With regard to the overall number of patients, the contamination rate at wound closure remained approximately 10%, while at emergency it was 28%.

In group P the two gram-negative strains had already been isolated at the start of surgery (1 was resistant to pefloxacin, the other untested), while among the 35 gram-positive bacteria, 13 were previously absent: 11 strains were different from those isolated previously and two were newly acquired by patients with formerly sterile samples.

In group C, eight of the 13 gram-negative bacilli recovered were acquired during surgery and were unknown previously. Among the 19 gram-positive isolates, six were newly acquired in the operating theatre, one of which was a *Clostridium* sp. Ten strains were different from those previously isolated and four were newly acquired by patients with formerly sterile samples.

Table 2 Organisms cultured from fracture sites at each stage of sampling in the pefloxacin (P) group and in the cefazolin-oxacillin (C) group

Species	At presentation to ED			At start of surgery			At wound closure			At infection			In drain aspirates		
	Group P ($n=101$)	Group C ($n=110$)	Total[a] ($n=211$)	Group P ($n=63$)	Group C ($n=59$)	Total[a] ($n=122$)	Group P ($n=37$)	Group C ($n=32$)	Total[a] ($n=69$)	Group P ($n=21$)	Group C ($n=27$)	Total[a] ($n=48$)	Group P ($n=20$)	Group C ($n=18$)	Total[a] ($n=38$)
Gram-negative bacteria	23	31	54	13	19	32	2	13	15	2	13	15	4	9	13
Acinetobacter spp.	5	8	13 (5)	4	5	9 (4)	1	2	3 (2)	0	1	1	2	0	2
Enterobacter spp.	7	12	19 (8)	4	7	11 (2)	1	5	6 (2)	0	5	5 (2)	2	6	8 (2)
Klebsiella spp.	1	0	1 (1)	0	0	0	0	1	1 (1)	0	2	2 (2)	0	0	0
Escherichia coli	0	1	1 (1)	0	1	1 (1)	0	1	1	0	2	2 (2)	0	0	0
Citrobacter spp.	0	1	1 (1)	0	1	1 (1)	0	0	0	0	1	1 (1)	0	0	0
Proteus mirabilis	0	0	0	1	0	1 (1)	0	0	0	0	0	0	0	0	0
Morganella spp.	0	0	0	1	0	1 (1)	0	0	0	0	0	0	0	0	0
Salmonella spp.	0	0	0	0	0	0	0	0	0	1	0	1 (1)	0	0	0
Serratia spp.	1	1	2 (1)	0	0	0	0	2	2	0	0	0	0	0	0
Pseudomonas spp.	8	5	13 (7)	3	2	5 (3)	0	1	1 (1)	1	2	3 (1)	0	2	2 (1)
Unidentified GNB	1	3	4 (3)	0	3	3 (3)	0	1	1 (1)	0	0	0	0	1	1 (1)
Gram-positive bacteria	78	79	157	50	40	90	35	19	54	19	14	33	16	9	25
Total staphylococci	50	60	110	33	27	60	20	10	30	15	9	24	10	5	15
MSSA	4	5	9 (3)	5	0	5 (1)	3	2	5 (1)	7	4	11 (1)	0	0	0
MRSA	0	0	0	0	0	0	0	0	0	4	1	5	0	0	0
MXSA	0	0	0	1	1	2	0	0	0	1	1	2 (1)	0	0	0
MSCNS	36	48	84 (20)	26	22	48 (13)	15	8	23 (4)	0	0	0	8	5	13 (1)
MRCNS	2	3	5 (3)	1	1	2 (1)	1	0	1	3	3	6 (1)	1	0	1
MXCNS	8	4	12 (2)	0	3	3	1	0	1	0	0	0	1	0	1
Total streptococci	14	4	18	6	4	10	6	4	10	3	2	5	2	2	4
D streptococci	8	1	9 (4)	3	2	5 (1)	4	2	6	1	2	3 (1)	0	2	2
G streptococci	2	2	4 (0)	1	1	2 (1)	1	0	1	1	0	1	0	0	0
Nongroupable streptococci	4	1	5 (0)	2	1	3	1	2	3 (1)	1	0	1 (1)	2	0	2 (1)
Bacillus spp.	13	15	28 (15)	9	9	18	8	4	12 (6)	1	1	2	3	2	5 (3)
Clostridium spp.	1	0	1 (1)	1	0	1	0	1	1	0	2	2	1	0	1
Candida spp.	0	0	0	1	0	1	1	0	1	0	0	0	0	0	0

[a] Numbers in parentheses indicate organisms isolated as part of mixed cultures

GNB, gram-negative bacilli; MSSA, methicillin-sensitive *S. aureus*; MRSA, methicillin-resistant *S. aureus*; MXSA, *S. aureus* of undetermined methicillin susceptibility; MSCNS, methicillin-sensitive coagulase-negative staphylococci; MRCNS, methicillin-resistant coagulase-negative staphylococci; MXCNS, coagulase-negative staphylococci of undetermined methicillin susceptibility

Table 3 Organisms cultured from fracture sites in patients with positive cultures at wound closure

Organism	Pefloxacin group ($n=30$)					Cefazolin group ($n=27$)				
	Wound closure[a]	Presentation to ED	Start of surgery	Drain aspirate	Infection	Wound closure[a]	Presentation to ED	Start of surgery	Drain aspirate	Infection
MSCNS	15 (2)	10	10	2		8 (1)	6	2		
MRCNS	1 (1)									
MXCNS	1 (1)									
MSSA	3 (2)	1				2 (1)		1		
Streptococcus spp.	6 (3)	1	3			4 (2)		2		
Bacillus spp.	9 (4)	4	2	1	1	4 (1)	3	3		1
Clostridium spp.						1 (1)				
Acinetobacter spp.	1		1			2 (2)				
Enterobacter spp.	1	1	1			5 (3)	2	1	3	3
Escherichia coli						1 (1)				
Serratia spp.						2 (1)	1			
Klebsiella spp.						1 (1)				
Pseudomonas spp.						1	1			
Unidentified GNB						1	1	1		
Candida spp.	1		1							

[a] Numbers in parentheses indicate strains not previously isolated from the patient

ED, emergency department; MSCNS, methicillin-sensitive coagulase-negative staphylococci; MRCNS, methicillin-resistant coagulase-negative staphylococci; MXCNS, coagulase-negative staphylococci of undetermined methicillin susceptibility; MSSA, methicillin-sensitive *S. aureus*; GNB, gram-negative bacilli

Gram-positive strains accounted for 16 of 20 (80%) positive cultures from drain aspirates in group P and nine of 18 (50%) in group C. All of these strains were coagulase-negative staphylococci (1 MRCNS in group P); there was no strain of *Staphylococcus aureus*. Nineteen of the 21 (90.5%) infecting strains in group P were gram-positive, compared to 14 of 27 (52%) in group C ($P<0.05$). In group P only two gram-negative strains were isolated. Fifteen of the 19 (78.9%) gram-positive bacteria isolated were staphylococci, with 12 of 15 (80%) being *Staphylococcus aureus* (4 MRSA and 1 untested). All coagulase-negative strains ($n=3$) were methicillin resistant.

In group C, the main species recovered among gram-negative bacilli were *Enterobacter* spp. (5/13, 38.5%), followed by *Klebsiella* spp., *Escherichia coli*, *Pseudomonas* spp., and *Acinetobacter* spp. Among gram-positive bacteria, nine of 14 (64.3%) isolates were staphylococci, mainly *Staphylococcus aureus* (6/9 staphylococci) (1 MRSA and 1 untested). Coagulase-negative strains ($n=3$) were methicillin resistant. Positive cultures in infected patients were significantly correlated with positive cultures at emergency, at the start of surgery, and at the end of surgery ($P<0.05$) but not with positives cultures from drain aspirates.

Infecting isolates are listed in Table 4. Nine of the 18 infected patients in group P had previously sterile samples. Previous cultures were positive with the same strain for only two of the nine patients. Among the 20 patients infected in group C, nine had no previous positive culture. In five of 11 cases, the strain had been isolated previously.

Slime Production and Adherence Properties. At the emergency department, 78 strains from group P and 85 from group C were available for testing slime production and adherence properties. There was no significant difference between strains with respect to slime production or adherence (Figure 1).

At wound closure, 27 strains from group P and 27 from group C were tested. Although slime-producing strains were equally represented, adherent strains were more often present in group P [16/27 (59.3%) vs. 7/21 (33.3%), $P=0.07$]. However, six of 27 (22%) strains in group P and four of 21 (19%) strains in group C exhibited both characteristics ($P=<0.05$).

In infected cases, 41.7% of the strains in group P and 25% in group C produced slime, and adherence was present in 50% and 31.5% of the strains, respectively ($P<0.05$ in both cases).

Resistance to Treatment. In group P, the rate of resistance to pefloxacin among strains isolated from drain tubes was 50% versus approximately 30% at emergency, at the start of surgery and at wound closure (Figure 2). In infected patients, 13 of 18 (72%) strains were resistant to pefloxacin, with ten of 18 (55.5%) strains also exhibiting a significant increase in resistance to cefazolin. In group C, cefazolin resistance was higher than pefloxacin resistance: 39 of 91 (42.8%) strains versus 16 of 96 (16.7%; $P<0.01$) were resistant at emergency, and similar proportions were found at the start of surgery and at wound closure. In drain aspirates, resistance to cefazolin was increased: ten of 15 (66.6%) strains were resistant; the same proportion of

Table 4 Organisms isolated at infection and at prior sampling times in the 38 infected patients

Patient no.	Organism isolated				
	At infection	At presentation to ED	At start of operation	At wound closure	In drain aspirate
Pefloxacin group ($n=18$)					
1	MXSA	no previous isolate			
2	MRSA	no previous isolate			
3	MSSA	no previous isolate			
4	MSSA	no previous isolate			
5	MRCNS	no previous isolate			
6	MSSA	no previous isolate			
7	MSSA	no previous isolate			
8	MSSA + nongroupable streptococci	no previous isolate			
9	MRSA	no previous isolate			
10	MSSA	*Pseudomonas* spp.	*Acinetobacer* spp. + MRCNS		
11	MSSA	MSCNS	*Bacillus* spp.		
12	D streptococci	*Pseudomonas* spp.			
13	*Pseudomonas* spp.	D streptococci	D streptococci	D streptococci	
14	MRSA	*Bacillus* spp. + *Pseudomonas* spp.			
15	*Bacillus* spp. + *Salmonella* spp.	*Bacillus* spp. + MSCNS	*Bacillus* spp.	*Bacillus* spp.	*Bacillus* spp.
16	MRSA	*Acinetobacter* spp.	MXSA	D streptococci	
17	MRCNS + MRCNS	*Acinetobacter* spp. + *Enterobacter* spp. + *Klebsiella* spp.		*Bacillus* spp.	MSCNS
18	G streptococci	MSCNS + MRCNS		G streptococci	
Cefazolin group ($n=20$)					
1	MRCNS	no previous isolate			
2	MSSA	no previous isolate			
3	*Klebsiella* spp. + *Escherichia coli*	no previous isolate			
4	MSSA	no previous isolate			
5	MRCNS	no previous isolate			
6	D streptococci + MXSA	no previous isolate			
7	*Pseudomonas* spp. + *Citrobacter* spp.	no previous isolate			
8	MRSA	no previous isolate			
9	D streptococci	no previous isolate			
10	*Bacillus* spp.	*Enterobacter* spp. + *Serratia* spp.	*Enterobacter* spp.	*Serratia* spp.	
11	*Pseudomonas* spp.	*Bacillus* spp. + *Enterobacter* spp. + *Pseudomonas* spp.	*Bacillus* spp. + XGNB		
12	*Escherichia coli* + *Klebsiella* spp. + *Enterobacter* spp.			*Enterobacter* spp.	*Enterobacter* spp. + XGNB
13	*Enterobacter* ssp.	*Enterobacter* ssp.	*Enterobacter* ssp.	*Enterobacter* ssp.	*Enterobacter* ssp.
14	*Acinetobacter* spp.	MSCNS + MSCNS + MRCNS			
15	MSSA	XGNB + *Bacillus* spp.	XGNB + *Bacillus* spp.	XGNB + *Bacillus* spp.	
16	MRCNS			MSCNS	
17	*Enterobacter* spp.	*Acinetobacter* spp.	MSCNS	*Enterobacter* spp.	
18	MSSA	*Enterobacter* spp.			
19	*Enterobacter* spp.	*Acinetobacter* spp. + D streptococci		*Serratia* spp.	
20	*Clostridium perfringens* + *Clostridium* spp. + *Enterobacter* spp.	*Pseudomonas* spp. + *Bacillus* spp. + *Enterobacter* spp.	*Bacillus* spp.	*Pseudomonas* spp. + *Bacillus* spp.	*Pseudomonas* spp. + *Bacillus* spp. + *Enterobacter* spp.

ED, emergency department; MXSA, *S. aureus* of undetermined methicillin susceptibility; MRSA, methicillin-resistant *S. aureus*; MSSA, methicillin-sensitive *S. aureus*; MRCNS, methicillin-resistant coagulase-negative staphylococci; NG *Streptococcus*, non-groupable streptococcus; XGNB, unidentified gram-negative bacillus; MSCNS, methicillin-sensitive coagulase-negative *S. aureus*

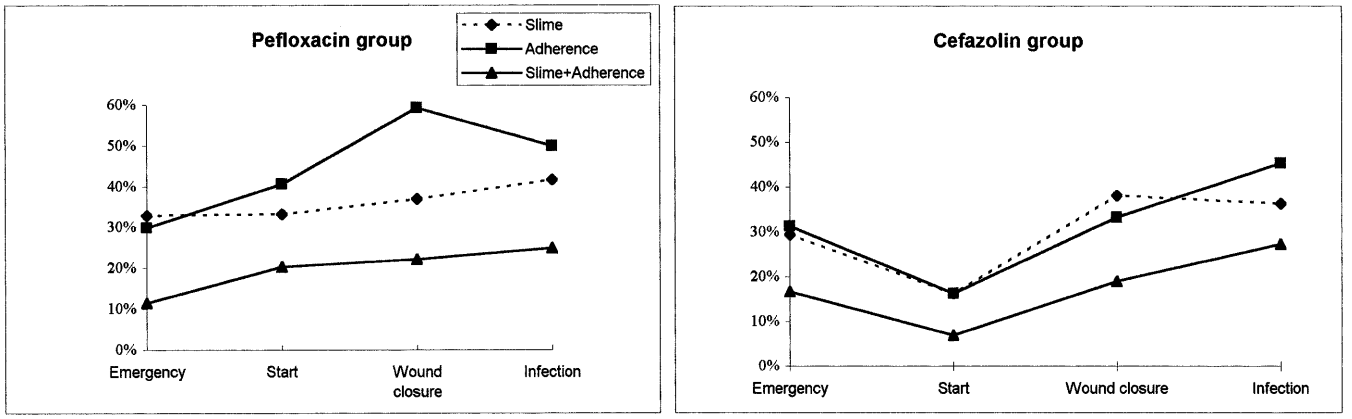


Figure 1 Proportions of strains with slime and/or adherence properties

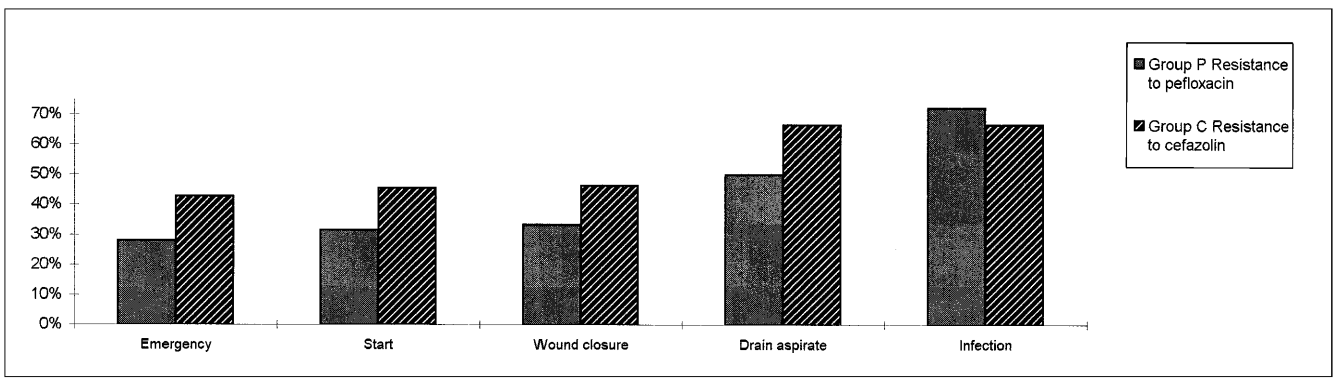
resistance found in infected patients. The increase in resistance to pefloxacin was moderate in this group: seven of 21 (33%) strains were resistant. Among staphylococci isolated at emergency, an increase in methicillin-resistant strains was observed, from 3.7 to 50% between the start of surgery and infection (Figure 3). Methicillin-resistant *Staphylococcus aureus* was found only in infected patients, while resistant strains found at other stages were coagulase-negative staphylococci. Among gram-negative bacteria, resistance to pefloxacin was present at emergency for two of 52 (3.8%) strains, at the start of surgery for six of 27 (22.2%) strains, and at wound closure for three of 12 (25%) strains tested. In infected patients three of 14 (21.4%) strains tested were resistant to pefloxacin. Only one strain of gram-negative bacteria was susceptible to cefazolin at emergency, at the start of surgery and at wound closure, and three of 14 strains were susceptible in infected patients.

Discussion

Numerous studies, most often retrospective, have been published on the subject of open fractures [6–8]. The best documented study is a prospective randomised (not double-blind) trial by Patzakis et al. [9] that included 238 patients with 255 open fractures who received no antibiotic or were treated for 10 days with penicillin plus streptomycin or cephalothin. Fractures were not graded according to severity. Rates of infection were 14%, 10%, and 2% ($P<0.03$). Patients receiving cephalothin, although less frequently infected, had a higher rate of gram-negative infecting bacilli. In this study, 65% of the four samples taken upon arrival, at the start of surgery, at the end of surgery, and subsequently were positive. The authors found that gram-negative bacilli prevailed in open fractures (60%), while staphylococci represented 40% of species isolated.

The results of our prospective study, which included 616 patients, appear to be different. The contamination rate at emergency was lower: only 26% of the samples in group P and 30% in group C were positive. At the end of surgery and in drain aspirates, contamination was mainly due to gram-positive bacteria. These results are not in agreement with previous studies in which gram-negative bacilli were more often recovered.

Figure 2 Overall resistance to pefloxacin and cefazolin-oxacillin at the various stages of sampling



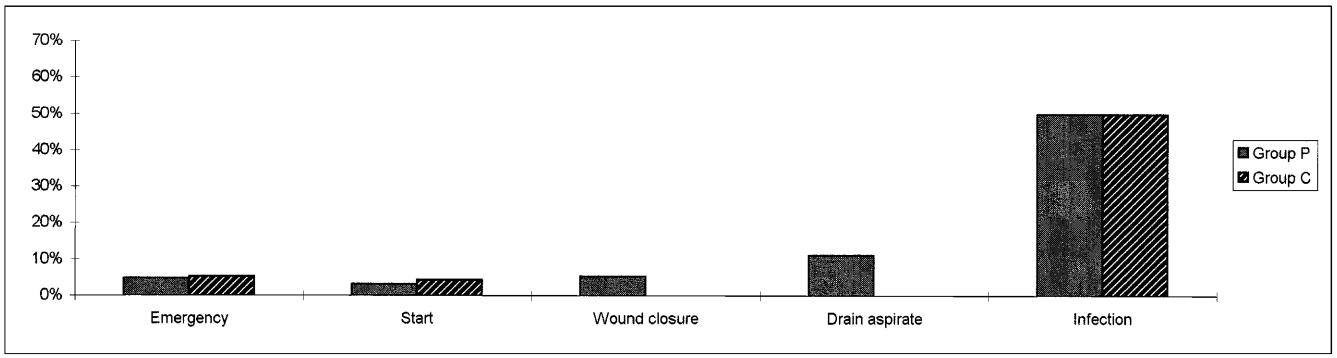


Figure 3 Methicillin resistance among staphylococci at the various stages of sampling

It is important to note that *Staphylococcus aureus* or methicillin-resistant strains of staphylococci were absent among the strains isolated at initial sampling stages, as observed by Dietz et al. [10]. Among gram-negative bacilli, species were equally represented in both treatment groups.

At emergency and at the start of surgery, there was a similar distribution of species in each treatment group, while at wound closure it was significantly different. In group P, gram-positive bacteria still prevailed, while gram-negative bacteria were present in group C. At this stage, most staphylococci isolated in either group remained methicillin susceptible. A comparison of the species present in the two treatment groups showed that 66% of the patients in each group whose cultures were positive at wound closure had harboured the same bacterial species at the emergency department and/or at the start of surgery. For 34% of patients, the species had not been isolated previously.

In infected patients, although similar proportions of gram-positive and gram-negative bacteria were observed in both treatment groups as those found at wound closure, the bacterial species isolated were different. In group P, 90.5% of the strains isolated were gram positive.

It is noteworthy that among the staphylococci isolated, most were *Staphylococcus aureus* (33% methicillin resistant), and both coagulase-negative strains isolated were methicillin resistant, as shown by Boxma et al. [11]. In group C, gram-negative bacteria accounted for 48.1% of the strains isolated. Among staphylococci, 66.7% were *Staphylococcus aureus*, and all coagulase-negative staphylococci were methicillin resistant.

Like Gustilo and Anderson [12], who used cephalosporins, we found that the infecting organisms were more frequently gram-negative bacteria in group C than in group P. In group C, one patient became infected with a *Clostridium* sp. even though he had received appropriate anti-anaerobe treatment.

Adherence is known to constitute the first step of bacterial colonisation, and anti-adhesive properties of antibiotics may be important in prophylaxis, as shown in an experimental model [13]. In a first study [14], we showed that for coagulase-negative staphylococci, slime production and adherence properties were predictive of their pathogenicity in relation to foreign material and in bone infections. Strains possessing one or both of these properties were found in approximately 50% of isolates at emergency and at the start of surgery, while this proportion was higher at wound closure. Strains in group P were more adherent at wound closure than those in group C. This discrepancy is probably related to the high proportion of gram-positive bacteria in group P, which are known to adhere better to foreign material. However, these results may be linked to the method used for determining adherence properties: testing adherence to cells rather than to inert material might have been more appropriate for gram-negative bacilli. In drain aspirates, 30% of the strains tested were adherent or slime producing. This result is surprising and suggests that adherent strains probably escaped isolation because they were undetectable in fluids, and it may also explain the poor correlation between species recovered in drain aspirates and those responsible for infection. This would plead in favour of drain tube cultures with adequate procedures to dislodge adherent bacteria [15].

In group P, no progression of resistance was noted among gram-negative bacilli between consecutive sampling stages. Among *Staphylococcus* spp. strains, 3% were methicillin resistant at the start of surgery, and 16.7% were resistant to pefloxacin. However, in infected patients, rates of resistance to methicillin and pefloxacin were 50% and 69%, respectively.

Gustilo and Anderson [12] found that in 77% of cases of infection following antibiotic prophylaxis with cephalosporins, strains resistant to these compounds were involved. In the present study, in group C, we also found increased resistance to cefazolin, both in drain aspirates and in case of infection, which is related to the

larger proportion of gram-negative bacilli isolated at those stages (48% vs. 28% at emergency). Among staphylococci, an increase in methicillin-resistant strains was observed, from 3.7 to 44.5%, between the start of surgery and infection.

Two patients in group P and five in group C were infected with the same species isolated at prior stages. Microorganisms recovered at infection were already present at wound closure in five of these patients. In infected patients who already had a positive culture at a prior stage, the sample taken at emergency was most often positive (18/20 cases) but was not predictive of the infecting species. Pathogens were isolated for the first time at infection in 18 of the 38 (47.4%) infected patients.

This randomised trial included 43 centres. Infected patients were distributed among various centres with no apparent cluster effect. The global contamination rate observed in this trial was lower than those described in the literature.

Distribution of gram-positive and gram-negative bacteria in grade 1 and 2 fractures was similar to that usually observed in cutaneous flora. At wound closure, in group P, gram-positive bacteria accounted for the vast majority of the organisms isolated, and the same proportions were found at infection, although the infecting strains were different (*Staphylococcus aureus* or methicillin-resistant coagulase-negative staphylococci), suggesting a probable nosocomial origin. In the group C, the proportion of gram-negative bacteria was higher at wound closure. Although the infecting strains were different, a similar distribution of bacteria was found in infected patients.

The similarity in the distribution of contaminating strains in both treatment groups at wound closure and in infected patients suggests the possibility of perioperative contamination and of the nosocomial origin of these strains. They were more often resistant to the antibiotic prophylaxis received by the patient but had not been cultured from prior samples. Adherence properties increased between emergency and wound closure. For infecting strains, this property tended to decrease in group P, expressing a change in trend.

The presence of bacteria at prior sampling stages may be predictive of infection [16]: positive cultures at the end of surgery correlated significantly with later infection ($P<0.01$); however, they do not appear to be indicative of the type of infecting species, even when strains have been isolated during surgery, just before wound closure. Cultures of drain aspirates had no predictive value ($P=0.07$).

Infectious complications after bone surgery are believed to result from strains acquired within the operating theatre, and the reason those strains were not cultured from samples taken at wound closure remains to be clarified.

Van Ogtrop [17] has commented that accurate detection of *Staphylococcus aureus* requires broth enrichment procedures, which may not have been used systematically in this trial, hence resulting in false-negative results. Biopsy samples, such as those used for burned patients, might have been more reliable.

Infection by gram-negative bacteria was noted in group C and not in group P. In the latter group, infection with staphylococci, in particular *Staphylococcus aureus* was observed. This difference might be related to pefloxacin's effectiveness against gram-negative bacteria but not against staphylococci in the slow-growth phase [18]. Prophylaxis with a single dose of pefloxacin resulted in an infection rate of 6.6%, while 5 days of cefazolin-oxacillin resulted in an infection rate of 8%. This difference is not significant, but there were too few patients to detect a difference.

In a study that included 2195 patients, Boxma et al. [11] found an infection rate of 3.3% after antibiotic prophylaxis with ceftriaxone, while in the placebo group, the rate was 8.3%. The difference was shown to be significant because of the larger number of patients and the presence of a placebo group. The absence of gram-negative bacilli, the reduction in adherent infecting strains, and easy compliance are in favour of pefloxacin for these grades of open fractures. However, the emergence of nosocomial methicillin-resistant staphylococci is not resolved and raises the problem of an associated antibiotic. Antistaphylococcal agents such as cefazolin and oxacillin do not appear to be sufficiently effective to prevent infection by these organisms.

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