ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Nov. 1998, p. 3002–3005 Vol. 42, No. 11
0066-4804/98/$04.00+0

Antimicrobial Susceptibility of Bacteria Isolated from Orthopedic Implants following Revision Hip Surgery

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Received 27 February 1998/Returned for modification 23 July 1998/Accepted 13 August 1998

The susceptibilities of 49 isolates recovered from orthopedic implants to seven antimicrobial agents were evaluated by the broth microdilution method. Ciprofloxacin and vancomycin were more active than gentamicin, representing aminoglycosides which are routinely incorporated into bone cement, and also more active than the peroperative antimicrobial agents cefamandole and erythromycin. The use of ciprofloxacin and vancomycin in vivo, therefore, warrants further evaluation.

Total hip replacement has become commonplace in recent years because of the success of this procedure in restoring function to the affected joint (6). Unfortunately, bacterial infection has been a significant complication following this procedure, with implant infection implicated in 22% of revision operations in a recent study (12). Removal and replacement of the prosthesis are usually required to eradicate the infection, with attendant patient trauma and increased cost (1, 8). Antibiotic treatment to reduce the risk of recurrent infection includes the use of antibiotic-impregnated bone cement for prosthesis fixation at revision surgery (3) and the intravenous administration of antibiotics during revision surgery. In Musgrave Park Hospital, Belfast, United Kingdom, gentamicin is incorporated into bone cement and the cephalosporin cefamandole (Kefadol) is used for routine antimicrobial prophylaxis. When patients undergoing revision hip surgery are allergic to cefamandole, erythromycin is usually employed prophylactically.

Gentamicin resistance among bacteria isolated from infected hip joints has been reported. In a study of 33 infected hip joints, Weber and Lautenbach (13) noted that 29% of bacteria isolated preoperatively were resistant to gentamicin. Interestingly, following the use of gentamicin-impregnated bone cement, resistance increased to 41% of bacteria isolated postoperatively. In another study of cemented total hip arthroplasty infection caused by coagulase-negative staphylococci (CNS), Hope et al. (5) reported that the use of gentamicin-impregnated cement in the primary arthroplasty was associated with the emergence of gentamicin-resistant CNS in subsequent infection. Of 34 hip implants at revision surgery in which gentamicin-impregnated cement had been used at the previous operation, 30 (88%) later grew at least one strain of gentamicin-resistant CNS. In contrast, of 57 hip implants at revision surgery in which gentamicin was not included in the bone cement, only 9 (16%) later grew gentamicin-resistant CNS. In addition, an earlier study to determine the efficacy of antimicrobial agents in eradicating the normal skin microbiota prior to surgery reported that 18 of 152 patients (12%) had cefamandole-resistant *Staphylococcus epidermidis*, leading the authors to conclude that preoperative antimicrobial prophylaxis with cefamandole would have failed to protect these patients from the *S. epidermidis* which colonized their skin (11). The aim of the present study was, therefore, to determine the susceptibilities of bacteria isolated from revision hip prostheses to the commonly used antimicrobial agents gentamicin, cefamandole, and erythromycin and also to a range of alternative antimicrobial agents.

Twenty-six of 120 implants removed consecutively from patients undergoing revision hip surgery at Musgrave Park Hospital during the 14-month period from March 1996 to May 1997 were diagnosed as infected (12). From these infected implants, 49 clinical isolates were recovered. Review of the hospital notes for 18 patients with culture-positive implants and 52 patients with culture-negative implants revealed that infection prior to revision was suspected in only 8 cases (11%). Implants from 6 of these patients (75%) were subsequently diagnosed as infected in our study. Seven of the implants were infected by a single *Staphylococcus* sp., and a further three were infected by a combination of two *Staphylococcus* spp. The anaerobic bacterium *Propionibacterium acnes* was isolated as the single infecting organism from 12 implants, and a further 4 implants were infected by a combination of *P. acnes* and a gram-positive coccus. The isolates comprised the following: *S. epidermidis*, 17 strains; *Staphylococcus aureus*, 4 strains; *Staphylococcus hominis*, 3 strains; *Staphylococcus capitis*, 2 strains; *Staphylococcus haemolyticus*, 2 strains; *Staphylococcus sciuri*, 1 strain; *Micrococcus* sp., 1 strain; and *P. acnes*, 19 strains.

The following antimicrobial agents were used: gentamicin sulfate, erythromycin, and fusidic acid (Sigma Chemical Co., Poole, Dorset, United Kingdom); cefamandole naftate as Kefadol (Dista Products Ltd., Basingstoke, United Kingdom); cefotaxime as Claforan (Roussel Laboratories Ltd., Uxbridge, United Kingdom); ciprofloxacin as Ciproxin (Bayer plc, Newbury, United Kingdom), and vancomycin as Vancocin (Eli Lilly and Company Ltd., Basingstoke, United Kingdom). MICs were determined by the broth microdilution method (9, 10). Serial twofold dilutions of each antimicrobial were prepared in cation-supplemented Mueller-Hinton broth (MHB with 50 mg of Ca^{2+} and 25 mg of Mg^{2+} per liter; Unipath Ltd., Basingstoke, United Kingdom) within dilution schemes of 0.5 to 1,024 μg/ml (gentamicin, cefamandole, cefotaxime, and erythromy-

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TABLE 1. Antimicrobial susceptibilities of bacteria isolated from orthopedic implants

Isolate (no. of strains tested)	Test agent	MIC (μg/ml)			% Susceptible	MBC (μg/ml)		
		Range	50%	90%		Range	50%	90%
All (49)	Gentamicin	<0.5–512	8	64		1–>1,024	32	1,024
	Cefamandole	<0.5–64	1	32		<0.5–>1,024	1	64
	Cefotaxime	<0.5–64	2	16		<0.5–>1,024	64	512
	Erythromycin	<0.5–>1,024	16	>1,024		<0.5–>1,024	256	>1,024
	Vancomycin	0.25–2	1	2		1–64	32	64
	Ciprofloxacin	0.125–2	0.5	1		0.125–64	8	32
	Fusidic acid	<0.125–32	1	8		1–>256	16	>256
Staphylococcus spp. (30)	Gentamicin	<0.5–512	16	128	26	1–>1,024	32	1,024
	Cefamandole	<0.5–64	2	64	63	1–512	16	128
	Cefotaxime	<0.5–32	4	16	77	4–>1,024	128	1,024
	Erythromycin	<0.5–>1,024	256	>1,024	6	2–>1,024	>1,024	>1,024
	Vancomycin	0.25–2	1	2	100	1–64	32	64
	Ciprofloxacin	0.125–2	0.5	1	100	0.125–64	16	32
	Fusidic acid	<0.125–16	0.25	16	NA[a]	1–>256	64	>256
P. acnes (19)	Gentamicin	<0.5–16	4	8	NA	2–128	8	64
	Cefamandole	<0.5	<0.5	<0.5	100	<0.5–4	<0.5	1
	Cefotaxime	<0.5–1	<0.5	<0.5	100	<0.5–128	<0.5	2
	Erythromycin	<0.5–>1,024	<0.5	>1,024	NA	<0.5–>1,024	<0.5	>1,024
	Vancomycin	<0.125–1	0.5	0.5	NA	4–>256	8	32
	Ciprofloxacin	0.5–1	1	1	NA	1–128	8	32
	Fusidic acid	<0.125–8	1	2	NA	2–>256	16	32

[a] NA, no MIC breakpoint approved by the National Committee for Clinical Laboratory Standards.

cin) and 0.125 to 256 μg/ml (vancomycin, ciprofloxacin, and fusidic acid). The microdilution trays were stored in sealed plastic bags at −70°C and used within 3 weeks.

The inoculum for facultative isolates to be tested was prepared by adjusting the turbidity of an actively growing broth culture in MHB to an optical density at 540 nm equivalent to 1×10^8 CFU/ml. The suspension was further diluted to provide a final inoculum density of 5×10^5 CFU/ml. Anaerobic isolates to be tested were grown on anaerobic horse blood agar plates at 37°C for 48 h in an anaerobic chamber (Don Whitley Scientific, Shipley, United Kingdom). The inoculum was prepared by suspending bacteria from these plates in prereduced MHB, which provided optimal growth conditions for the *P. acnes* isolates. The suspension was then adjusted by spectrophotometric measurement to provide a final inoculum density of 10^6 CFU/ml.

The microdilution trays were removed from the freezer and thawed, and trays to be used for anaerobic bacteria were equilibrated in the anaerobic chamber for 4 h. The final inoculum (100 μl) was added to each well of the microdilution trays. Facultative isolates were incubated in air at 37°C for 24 h, and the anaerobic *P. acnes* isolates were incubated in the anaerobic chamber at 37°C for 48 h. After incubation, the MIC was read as the lowest concentration of each antimicrobial agent which inhibited visible growth of the test isolate. Quality assurance testing was performed with *Enterococcus faecalis* ATCC 22697 and *Bacteroides fragilis* ATCC 25285.

In order to determine the minimum bactericidal concentration (MBC), 20-μl aliquots were inoculated onto Mueller-Hinton agar plates which were incubated as described previously. The MBC was defined as the lowest antibiotic concentration that produced greater than 99.9% killing of the initial inoculum.

The results of this study are summarized in Tables 1 and 2. Control strains gave reproducible results, with MICs within National Committee for Clinical Laboratory Standards limits and 1 dilution of the mean. The majority of facultative isolates were resistant to gentamicin and erythromycin. In contrast, there was less resistance of facultative isolates to cefamandole, cefotaxime, and fusidic acid. Vancomycin and ciprofloxacin were most effective against the facultative isolates. All *P. acnes* strains were susceptible to cefamandole, cefotaxime, vancomycin, ciprofloxacin, and fusidic acid. However, higher concentrations of both gentamicin and erythromycin were required to inhibit the *P. acnes* strains. Based on overall MBCs at which 90% of strains tested were killed, ciprofloxacin was the most active bactericidal agent tested, followed in decreasing order by cefamandole, vancomycin, cefotaxime, gentamicin, fusidic acid, and erythromycin.

Although higher antibiotic concentrations are achieved locally with antibiotic-impregnated bone cement (4), this in vitro study has shown by the high numbers of gentamicin-resistant bacteria which were isolated that the routine use of gentamicin-impregnated bone cement may be ineffective. This finding was not unexpected as virtually all the retrieved implants had been fixed in place with gentamicin-impregnated bone cement, and it supports the results previously reported by Weber and Lautenbach (13). The use of erythromycin peroperatively in patients who are allergic to cephalosporins may also be ineffective, based on the high proportion of erythromycin-resistant bacteria isolated. The results described herein suggest that the use of other agents, for example, vancomycin and ciprofloxacin, in bone cement and peroperatively, respectively, could be more effective for the elimination of implant infection at the time of revision hip surgery and for the prevention of further implant infection. Previous studies have reported that the stability and physicochemical properties of vancomycin are not adversely affected by its addition to bone cement (7) and have also shown that the drug is released in sufficient concentrations to treat and prevent experimentally induced *S. aureus* osteomyelitis in rats (2). Further work to determine the efficacy of these antibiotics against bacteria growing within adherent biofilms on the surface of implant biomaterials is under way.

TABLE 2. Antimicrobial susceptibilities of staphylococcal species isolated from orthopedic implants

Isolate (no. of strains tested)	Test agent	MIC (μg/ml)			MBC (μg/ml)		
		Range	50%	90%	Range	50%	90%
S. epidermidis (17)	Gentamicin	<0.5–512	16	256	1–>1,024	128	>1,024
	Cefamandole	<0.5–64	4	32	1–512	16	64
	Cefotaxime	<0.5–32	4	16	4–>1,024	128	512
	Erythromycin	<0.5–>1,024	>1,024	>1,024	2–>1,024	>1,024	>1,024
	Vancomycin	1–2	2	2	8–64	16	64
	Ciprofloxacin	0.25–1	0.5	1	0.5–64	16	32
	Fusidic acid	<0.125–16	0.5	16	1–>256	>256	>256
S. aureus (4)	Gentamicin	16–32			16–32		
	Cefamandole	32			64–512		
	Cefotaxime	2–4			256–>1,024		
	Erythromycin	2–16			2–>1,024		
	Vancomycin	0.5–1			16–32		
	Ciprofloxacin	0.5–1			1–32		
	Fusidic acid	<0.125–0.25			16–128		
S. hominis (3)	Gentamicin	<0.5–32			2–64		
	Cefamandole	1–64			1–>1,024		
	Cefotaxime	2–4			64–>1,024		
	Erythromycin	128–>1,024			1,024–>1,024		
	Vancomycin	1			16–32		
	Ciprofloxacin	0.25–2			0.25–32		
	Fusidic acid	0.25–32			4–>256		
S. capitis (2)	Gentamicin	<0.5–16			8–64		
	Cefamandole	1			1–16		
	Cefotaxime	2–4			128–512		
	Erythromycin	256			256–>1,024		
	Vancomycin	1			32		
	Ciprofloxacin	0.25			8–16		
	Fusidic acid	<0.125–0.25			32–64		
S. haemolyticus (2)	Gentamicin	64			256–512		
	Cefamandole	32–64			64		
	Cefotaxime	32–64			64–256		
	Erythromycin	32–>1,024			>1,024		
	Vancomycin	1–2			1–32		
	Ciprofloxacin	0.25			8–16		
	Fusidic acid	1–8			4–>256		
S. sciuri (1)	Gentamicin	4			16		
	Cefamandole	1			1		
	Cefotaxime	4			64		
	Erythromycin	64			>1,024		
	Vancomycin	1			32		
	Ciprofloxacin	0.5			32		
	Fusidic acid	0.25			>256		
Micrococcus sp. (1)	Gentamicin	16			32		
	Cefamandole	1			8		
	Cefotaxime	2			256		
	Erythromycin	>1,024			>1,024		
	Vancomycin	0.25			1		
	Ciprofloxacin	2			2		
	Fusidic acid	<0.125			4		

The technical assistance of Stef McGrath, School of Pharmacy, The Queen's University of Belfast, is gratefully acknowledged.

Michael Tunney and these investigations were funded by the Arthritis Research Campaign, UK (project grant number P0522); Gordon Ramage was funded by a Department of Education for Northern Ireland research studentship.

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