Antimicrobial Susceptibility of *Propionibacterium acnes* and Related Microbial Species

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The minimal inhibitory concentrations of 32 antimicrobial agents were established for 73 strains of *Propionibacterium acnes* and four related species (P. granulosum, P. avidum, Corynebacterium minutissimum, and C. parvum). Most strains showed good susceptibility to those agents usually considered active against gram-positive organisms. With the exception of C. minutissimum, the strains tested revealed more or less identical susceptibility ranges. The lowest minimal inhibitory concentrations were observed with benzylpenicillin, ampicillin, cephalothin, rifampin, erythromycin, clindamycin, and minocycline. C. minutissimum was more susceptible to gentamicin, sisomicin, tobramycin, and fusidic acid but more resistant to most other drugs than were the other species examined.

Microaerophilic propionibacteria may be regarded not only as normal inhabitants of the skin but also as facultative, pathogenic organisms. Propionibacterium acnes can regularly be isolated from acne vulgaris lesions (14) and frequently from actinomycotic (9) or nonspecific anaerobic infectious processes (13). There seems to be strong evidence that P. acnes is an important etiological factor in acne vulgaris because of the release of large amounts of free fatty acids from sebum triglycerides (16). Furthermore, in acne vulgaris only those antimicrobial agents that are able to attack P. acnes appear to be therapeutic (6). For this reason, information about the antibiotic susceptibility of P. acnes is needed, yet few reports have appeared to date (2, 3, 7, 8, 15). The present study was undertaken, therefore, to evaluate the susceptibility of 73 strains of P. acnes and related organisms to 32 antimicrobial drugs.

MATERIALS AND METHODS

Bacterial strains. A total of 73 well-defined strains of microaerophilic propionibacteria and corynebacteria were examined. These strains belonged to the following species.

(i) *P. acnes* (38 strains). Twenty strains of *P. acnes* were received from abroad: American Type Culture Collection (ATCC), strains 6919, 6921, 6922, 6923, and 11827; V. R. Dowell, Jr., Center for Disease Control, Atlanta, Ga., strains 5159, 6949, 6981, 6994, and 7010; J. G. Voss, The Proctor & Gamble Co., Cincinnati, Ohio, strains MC, A, A3, C45, C55, 174V, 0391, and UCLA18; C. S. Cummins, Anaerobe Laboratory, Virginia Polytechnic Institute and State University, Blacksburg, strains 162 and 3706. Eighteen strains of *P. acnes* were isolated in our own

laboratory from human hair or from infectious processes.

(ii) **P.** granulosum (15 strains). Ten strains of *P.* granulosum were cultivated in our institute and five strains were received from J. G. Voss (strains C51, D21, D34, and V1) or from C. S. Cummins (strain 0507).

(iii) **P.** avidum (16 strains). In Cologne we isolated 14 strains of *P. avidum*. Two additional strains were supplied by C. S. Cummins (strains 0575 and 0589).

(iv) Corynebacterium minutissimum (3 strains). Strains 22347, 22348, and 22349 were from ATCC.

(v) C. parvum (one strain). The only C. parvum strain tested was the ATCC strain 11829.

All strains were differentiated according to *Bergey's Manual of Determinative Bacteriology* (1); most had been used in previous published studies (4, 11, 12).

Media. For primary isolation, agar containing 0.43 M glycerol was used (5). Further cultivation was done on A-agar medium, the contents of which have been described by Jong et al. (4). Bacterial suspensions were prepared in Mueller-Hinton broth (Difco), and the antimicrobial drugs tested were added to Mueller-Hinton medium (Difco, pH 7.2).

Preparation of inocula. Cells from 48-h-old cultures on A-agar medium were added to Mueller-Hinton broth, carefully shaken to establish a homogeneous suspension, and brought to an extinction of 0.8 (measured at 540 nm against Mueller-Hinton broth using the Beckman photometer 1211). One drop (Pasteur pipettes) of each suspension was used as the inoculum. Based on the counts of the colonyforming units (CFU) that were done at least twice with one strain of each species, the following final inocula were calculated to be: *P. acnes* = 4 × 10⁶ CFU, *P. granulosum* = 1 × 10⁷ CFU, *P. avidum* = 3.1×10^7 CFU, *C. minutissimum* = 1.2×10^7 CFU, and *C. parvum* = 7 × 10⁶ CFU. For trimethoprim, sulfamethoxazole, and the combination of these two drugs (1 + 19 parts), subculturing was done on Mueller-Hinton medium. The strain suspension of *P. avidum* and of the remaining strains was diluted 1:100 and 1:10, respectively, in distilled water.

Antimicrobial agents. A total of 33 preparations of 32 drugs were used for the antimicrobial agents. Potassium benzylpenicillin, sodium oxacillin, sodium dicloxacillin, sodium ampicillin, disodium carbenicillin, streptomycin monosulfate, sisomicin sulfate, and chloramphenicol were supplied by Bayer AG, Leverkusen; sodium cephalothin, cephalexin monohydrate, sodium cefazolin, and tobramycin sulfate were from Eli Lilly GmbH, Giessen; kanamycin monosulfate, colistin methanesulfonate sodium, and tyrothricin were from Gruenenthal GmbH, Stolberg; gentamicin sulfate was from Merck, Darmstadt; tetracycline hydrochloride, rolitetracycline, lincomycin hydrochloride-monohydrate, and sodium novobiocin were from Hoechst AG, Frankfurt; oxytetracycline hydrochloride and doxycycline were supplied by Pfizer GmbH, Karlsruhe; chlortetracycline hydrochloride and minocycline monohydrochloride were from Lederle-Cyanamid GmbH, Munich; clindamycin hydrochloride was from Upjohn GmbH, Heppenheim; erythromycin glucoheptonate was from Schering AG, Berlin; fusidic acid was from Thomae GmbH, Biberach; sodium rifampin was from Ciba-Geigy AG, Wehr; bacitracin was from Dieckmann GmbH, Bielefeld; neomycin B hydrochloride was from Troponwerke, Cologne; and trimethoprim and sodium sulfamethoxazole were from Hoffmann-LaRoche AG, Grenzach.

All products were of known potencies and were dissolved and diluted according to the manufacturers' instructions.

Susceptibility tests. Minimal inhibitory concentrations (MICs) were determined using an agar dilution technique. Freshly prepared drug-Mueller-Hinton agar mixtures were poured into petri dishes. After solidification, the plates were dried for 3 h at 37°C and then inoculated immediately. Plates were incubated for 48 h at 37°C using the Heraeus anaerobic incubator VT/N₂ (95% N₂ and 5% CO₂ at normal atmospheric pressure), and the results were read immediately thereafter. The MIC was considered to be the lowest drug concentration in which no visible growth occurred. Antimicrobial activity controls with *Staphylococcus aureus* (strain SG 511-Jena) as well as growth controls of the strains tested, on drug-free medium, were included with each assay.

RESULTS

In Table 1 the MICs of eight β -lactam antibiotics are shown. Benzylpenicillin was the most active drug, and at 0.1 μ g/ml it inhibited 70 of the 73 strains tested. Cephalothin proved to be the best cephalosporin and compared favorably with ampicillin. The three *C. minutissimum* strains showed the least susceptibility to the penicillins, but this was not the case with the cephalosporins. Nevertheless, it can be stated that all the organisms tested were more or less penicillin- and cephalosporin-susceptible.

The activity range of six aminoglycosides examined is summarized in Table 2. Gentamicin showed the best MIC values, whereas kanamycin was the least active aminoglycoside tested. The observation was made that the three C. *minutissimum* strains tested were highly susceptible to some of these agents.

In Table 3 the MICs of six tetracyclines are shown. Some differences in the susceptibility of the 73 strains studied were noted. Chlortetracycline was the least effective in vitro, whereas minocycline was the most active. The MICs of the others were fairly comparable.

Of the antibiotics presented in Table 4, erythromycin and clindamycin exhibited the best activity. The activity of lincomycin was clearly inferior to that of clindamycin, as can be seen best with the *C. minutissimum* strains tested. Novobiocin and fusidic acid were able to attack *P. acnes* strains, too, but their activity range was poor. It is of interest that the three *C. minutissimum* strains examined were particularly susceptible to fusidic acid.

The results in Table 5 show that all strains tested were susceptible to chloramphenicol and bacitracin and resistant to colistin. Rifampin attracts attention because of its high activity against P. acnes, P. granulosum, P. avidum, and C. parvum; on the other hand, all three C. minutissimum strains showed marked resistance to rifampin. The activity of sulfamethoxazole was poor but, in combination with subinhibitory amounts of trimethoprim, greater activity seemed apparent. Although P. acnes, P. avidum, and the single C. parvum strain tested were susceptible to 5% trimethoprim plus 95% sulfamethoxazole, the activity of this drug combination towards P. granulosum and C. minutissimum was low.

DISCUSSION

Little information about the antibiotic susceptibility of *P. acnes* was found in previous publications. In 1942 Craddock (3) stated that the "acne bacillus" was less susceptible to penicillin than *S. albus*. It is not only difficult to compare the results reported by Craddock with our own observations, but also the present resistance pattern of *S. epidermidis* is quite different (10). Smith and Waterworth (15) reported the MICs of benzylpenicillin and tetracycline against 32 *P. acnes* strains; all strains were inhibited by 0.4 μ g of benzylpenicillin per ml and 94% were inhibited by 1.6 μ g of tetracy-

Antibiotio	Species		N	No. of s	trains i	nhibite	d at an	MIC (µ	g/ml) o	of:	
Antibiotic	Species	12.5	6.25	3.1	1.6	0.8	0.4	0.2	0.1	0.04	≤0.02
Benzylpeni-	P. acnes	_	_	_	_	_	·	_	_	35	3
cillin	P. granulosum	_	_	_	_	_			6	9	_
	P. avidum	-	-	_	_	-	_	_	4	12	_
	C. minutissimum			_	_	_	2	1	-	_	_
	C. parvum	-	-	_	-	-	-	-	-	1	-
Oxacillin	P. acnes	_	_	_	_	1	15	17	5	_	_
	P. granulosum	-	-	-	-	7	8		-	-	-
	P. avidum	-	-	-	-	5	8	3	-	-	_
	C. minutissimum	-	-	3	-	_	_	_	-	-	-
	C. parvum	-	-	-	-	-	-	1	-	-	_
Dicloxacillin	P. acnes	_	_	_	_	1	10	24	3	_	_
	P. granulosum	-	-	-	6	9	_	-	—	-	—
	P. avidum	-	-	-	6	7	2	1	_	_	-
	C. minutissimum	-	3		-	-	-	-	-	-	_
	C. parvum	-	-	-	-	1	-	-		-	-
Ampicillin	P. acnes	_	_	_	_	_	_	1	25	10	2
	P. granulosum	-	-	-	-	-	-	-	15	-	
	P. avidum	-	-	-	-	_	-	3	11	2	—
	C. minutissimum	-	-	-	-	3	-	-	-	-	-
	C. parvum	-	-	-	-	-	-	-	1	-	-
Carbenicil-	P. acnes	-	_	_	-	7	26	5	_	_	_
lin	P. granulosum	-	-	_	12	3	-	-	-	-	-
	P. avidum	-	-	-	6	8	2	-	-	-	-
	C. minutissimum	-	3	-	-	_	-	-	-		_
	C. parvum	-	-	-	-	1	-	-	-	-	-
Cephalothin	P. acnes	-	—	_	-	_	-	2	35	1	_
	P. granulosum	-	-	-	-	1	1	8	5	-	-
	P. avidum	-	-	-	-	-	5	7	4	-	_
	C. minutissimum	-	-	-	_	_	_	3	_	-	-
	C. parvum	-	-	-	-	1	-	—	-	-	-
Cephalexin	P. acnes	-	-	_	-	10	25	3	—	-	-
	P. granulosum	1	2	6	5	1	-	-	-	-	-
	P. avidum	1	-	-	-	11	4	-	-	-	_
	C. minutissimum	-	_	_	-	3	-	-	-	—	-
	C. parvum	-	1	-	-	-	-	-	-		-
Cefazolin	P. acnes	-	-	_	_	_	5	30	3	-	-
	P. granulosum	—	_	-	12	3	_		-	-	_
-	P. avidum	-	-	-	-	1	13	2	_	-	-
	C. minutissimum	-		-	_	-	3	-	-	_	-
	C. parvum	-	_	-	1	-	-	-	-	_	-

TABLE 1. Susceptibility of P. acnes and related species to β -lactam antibiotics (absolute numbers,
73 strains examined)

	. .		N	lo. of st	rains ir	hibited	at an I	MIC (µ	g/ml) of	: :	
Antibiotic	Species	≥100.0	50.0	25.0	12.5	6.25	3.1	1.6	0.8	0.4	0.2
Streptomycin	P. acnes	_	_	_	14	23	1	_	-	_	-
	P. granulosum	_	_	_		9	6	_	_	_	-
	P. avidum	_	_	_	2	14	-	_	-	_	_
	C. minutissimum		-		_	3	_	_		_	_
	C. parvum	-	-	-	-	1	-	-	—	-	-
Kanamycin	P. acnes	2	10	22	4	_	_		_	_	_
·	P. granulosum	1	3	11	_	<u> </u>	_	_		_	-
	P. avidum	16	_	_	_	_	_	_		_	-
	C. minutissimum	_	-	3	_	-	_	_	_	-	-
	C. parvum	-	-	1	-	-	-	-	-	-	-
Neomycin	P. acnes	_	_	22	12	4	_	_	_	_	_
•	P. granulosum	_	_	7	1	4	2	1	_	_	_
	P. avidum	_	_	16	_	_	_	_	_		_
	C. minutissimum	-	_	_	3	_	_	_	_	-	_
	C. parvum	_	-	1	-	-	-	-	-	-	-
Gentamicin	P. acnes	_	_	_	_	3	24	11	_	_	_
	P. granulosum	_	_	_	-	-	_	4	7	4	_
	P. avidum	_	_	_	-	2	14	-	-		_
	C. minutissimum	_		_	_	_	_	_	-	3	-
	C. parvum	—	— ·	-	-	-	-	-	1	-	-
Tobramycin	P. acnes	_	_	1	7	_	29	1		_	_
•	P. granulosum	_			1	_	11	2	1	_	
	P. avidum	_	15	1	_	_	_	_	_		_
	C. minutissimum	_	_	_	_	_	_	_	2	1	-
	C. parvum	_	-	-	-	1	-	-	-	-	-
Sisomicin	P. acnes	_	_	13	19	2	4	_	_	_	_
•	P. granulosum	-	9	5	_	-	1	-	—	-	-
	P. avidum	-	1	13	1	1	—	—	-	-	-
	C. minutissimum		-	-	-	-	-	-	-	-	3
	C. parvum	_		1	-	-	_	-	-	-	-

 TABLE 2. Susceptibility of P. acnes and related species to aminoglycoside antibiotics (absolute numbers, 73 strains examined)

Antibiotic	9i		1	No. of s	trains i	nhibite	d at an	MIC (µ	ug/ml) o	f:	
Antibiotic	Species	12.5	6.25	3.1	1.6	0.8	0.4	0.2	0.1	0.4	≤0.02
Tetracycline	P. acnes			9	13	12	2	1	1	_	_
	P. granulosum	_	1	_	5	8	1	_	_	_	_
	P. avidum	1	1	5	9	_	_		_	_	_
	C. minutissimum	_	_	_	3	_	_	_	<u> </u>	_	_
	C. parvum	-	—	-	1	-	-	-	-	-	-
Oxytetracy-	P. acnes	_	_	8	30	_	_	_	_	_	_
cline	P. granulosum	_	_		6	6	3	_			_
	P. avidum	-	_	5	11	_	_	_	_	_	_
	C. minutissimum	_	-	_	_	3	_		_		
	C. parvum	_	-	-	-	1	-		-	-	-
Chlortetra-	P. acnes	_	9	7	13	4	4	1	_	_	_
cycline	P. granulosum	_	_	4	6	1	1	1	2	_	_
-	P. avidum	1	12	3	_	_	_	_			_
	C. minutissimum	_	3	_	_	_	_	_	_	_	_
	C. parvum	-	-	-	1	-	-	-	-	-	-
Rolitetracy-	P. acnes	_	_	_	1	19	18	_	_	_	_
cline	P. granulosum	_	_	-	_	12	3	_	_		_
	P. avidum		_	_	5	9	2	_	_	_	
	C. minutissimum		_	_	_	3		_	_	_	-
	C. parvum	_	-	-	-	1	-	-	-	-	-
Doxycycline	P. acnes	_	_	1	27	10	_	_	_	_	_
	P. granulosum	_	_	3	4	6	2	_	_	_	_
	P. avidum	_	_	_	3	13	_	_	_	_	_
	C. minutissimum	_	_	_	_	3	_	_	_	-	-
	C. parvum	-	-	-	-	1	-	-	—	_	-
Minocycline	P. acnes	_	_	_	_	_	8	22	5	3	_
-	P. granulosum	-	-	_	_	_	<u> </u>	7	6	1	1
	P. avidum	_		_	_	_	14	2	_	_	-
	C. minutissimum	_	_	_	_	_	3	_	_	_	_
	C. parvum	-	-	-	-	-	-	1	-	-	-

 TABLE 3. Susceptibility of P. acnes and related species to tetracyclines (absolute numbers, 73 strains examined)

A	O sector		No.	of strai	ns inhil	bited at	an MIC	C (µg/m	l) of:	
Antibiotic	Species	6.25	3.1	1.6	0.8	0.4	0.2	0.1	0.4	≤0.02
Lincomycin	P. acnes		_		1	10	25	2	_	_
	P. granulosum	1	_		-	3	8	3	_	_
	P. avidum	_		_	_	9	7	_	_	_
	C. minutissimum	3	_	_	_	_	_	_	-	_
	C. parvum	-	-	-	-	-	1	-	-	-
Clindamycin	P. acnes	_	_	_	_	2	3	20	13	_
•	P. granulosum	_	_	_	_	_	1	1	12	1
	P. avidum	_	-	_	_	3	2	2	9	_
	C. minutissimum	_	_	-	_		3	_		
	C. parvum	-	-	-	-	-	1	-	-	-
Novobiocin	P. acnes	_	17	15	6	_	_	_	_	_
	P. granulosum	3	8	2	2		_		_	_
	P. avidum	4	1	5	3	3	_	-	_	_
	C. minutissimum	_	3	_	_		_		_	
	C. parvum	1	-	-	_	-	-	-	-	-
Erythromycin	P. acnes	_	_	_	_	·	_	2	22	14
	P. granulosum	_	_	_	_	_	_	_	_	15
	P. avidum	_	-	_	_	_	_	_	16	_
	C. minutissimum	_	_		-	1	2	_	_	_
	C. parvum	-	-	-	-	-	-	-	-	1
Fusidic acid	P. acnes	2	33	3	_	_	_	_	_	_
	P. granulosum	_	2	12	1	_	_	_	_	_
	P. avidum	1	15	_	_	_	-	-	_	_
	C. minutissimum		_	_	_	_	_	1	2	_
	C. parvum	_		_	1		_	_	_	

 TABLE 4. Susceptibility of P. acres and related species to lincomycin, novobiocin, erythromycin, and fusidic acid (absolute numbers, 73 strains examined)

Antibiotio	Section				No. o	f strai	ns inh	ibited	at an	MIC	(µg/ml) of:			
Antibiotic	Species	>100.0	100.0	50.0	25.0	12.5	6.25	3.1	1.6	0.8	0.4	0.2	0.1	0.04	≤0.02
Chloram-	P. acnes	_	_	_	_	_	-	1	33	4	_	-	_	_	-
phenicol	P. granulosum	_	_	_	_	-	-	_	3	9	3	-	-	_	-
-	P. avidum	_	_	-	_	_	_	1	15	_	_	- 1	_	_	_
	C. minutissimum	_	_	_	_		3	_	_	-	_	-	-	-	_
	C. parvum		-	-	-	-	-	-	-	_	1	-	-	-	-
Colistin	P games		1	22	15			_	_	_			_		_
Constan	P granuloeum		1		6	9									_
	P avidum		14	2	l _	_	_			_	_	_	l _	_	_
	C minutissimum	_	3		_	_	_	_	_	_	_	_		_	_
	C. parvum	_	_	-	-	1	-	-	-	-	-	-	-	-	-
Rifemnin	P cones											1	_	10	97
Mampin	P granulosum	_	_	_		_	_			_	_			10	15
	P avidum									_	_			_	16
	C minutieeimum	1	1				_	_			_				-
	C. parvum	_	_			_	-	_	_	_	_	_		_	1
	o i pai cant														_
Bacitracin	P. acnes	- 1	_	-	_	_	—	-	-	_	12	25	1	-	-
	P. granulosum	-	-	-	-	_	_	-	-	-	7	6	1	1	-
	P. avidum	-	-	-	-	_	-	10	6	-	-	-	-	-	-
	C. minutissimum	-	-	-	-	-	-	-	-		3	-	-	-	
	C. parvum	-	-	-	-	-	-	-	-	-	-	1	-	-	-
Tyrothricin	P. acnes	_	_	_	-	_	_	18	16	4	_	-	_	_	-
•	P. granulosum	-		-	_	4	4	7	-	_	-	-	-	-	-
	P. avidum	_	_		4	9	3	_	-	_	-	-	-	-	-
	C. minutissimum	-	-	-	3	-	-	-	-		-	-	-	-	-
	C. parvum	-	-	-	1	-	-	-	-	-	-	-	-	-	-
Trimetho-	P. acnes	_	_	_	_	_	_	_	-	18	18	2	_	-	_
prim	P. granulosum	_	_	- 1	-	1	4	4	4	_	_	2	-	_	_
	P. avidum	_	_	-	_	-	-	2	3	9	2	-	-	-	-
	C. minutissimum	-	-	2	-	1	-	-	-	-	-	-	-	-	-
	C. parvum	-	-	-	-	-	-	-	-	-	1	-	-	-	-
Sulfamethox-	P. acnes	38	_	_	_	_	_	_		_	_	-	_	_	_
azole	P. granulosum	14	_	1	-	_	_	_	l _	_	_	-	-	_	_
	P. avidum		_	Ĩ	1	2	4	8	_	-	_	_	_	-	_
	C. minutissimum	3		_	_	_	_	_	_	_	_	- 1	_	_	_
	C. parvum	1	-	-	- 1	-	-	-	_ ·	-	-	-	-	-	-
5% Trimetho	P. acnes	_	_	_	_	_	_	_	_	9	24	5	_		_
prim-95%	P. granulosum	_	_	_	-	3	1	6	4		_	_	_	_	_
sulfame-	P. avidum	_	_	_	_	<u> </u>	<u> </u>		3	6	7	- 1	-	_	_
thox-	C. minutissimum	- 1	_	1	_	2	_	-	_	-	_	-	_	_	-
azole	C. parvum	-	-		-	-	-	-	-	-	-	1	-	-	-

Table	5.	Susceptibility of P.	acnes and	related	species to	miscellaneous	antibiotics	(absolute	numbers,
				73 stra	ins exami	red)			

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cline per ml. Pochi and Strauss (8) examined 12 P. acnes strains and found benzylpenicillin, tetracycline, oxy- and chlortetracycline, novobiocin, erythromycin, and chloramphenicol to be very active, but only 17% of the strains were inhibited by 100.0 μg of neomycin per ml. Bordáčová and Výmola (2) performed susceptibility studies with seven antibiotics. All P. acnes strains tested were inhibited by 0.8 μ g of ampicillin per ml, 1.6 μ g of erythromycin per ml, and 0.2 μ g of bacitracin per ml, but only 13% were inhibited by 3.1 μ g of oxytetracycline per ml and 73% were inhibited by 6.2 μ g of chloramphenicol per ml. Martin et al. (7) established in 1972 MICs of 10 antibiotics against 16 P. acnes strains and found that benzylpenicillin, cephalothin, tetracycline, chloramphenicol, erythromycin, rifampin, and lincomycin were highly active but that the aminoglycoside antibiotics were only poorly active. Our data confirm and extend these observations.

The results of our study with 32 drugs demonstrate that these drugs offer a broader spectrum of activity against P. acnes and the related species tested than was ever observed with previously tested drugs. This fact should be taken into consideration when chemotherapy of infections with these organisms must be planned.

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