

Abstract

Background. Although *Staphylococcus aureus* and coagulase-negative staphylococci are the most common organisms isolated from bone and joint infections, other bacteria are occasionally found in these infections. Among bacteria found on the skin, *Propionibacterium* spp., although sometimes contaminants, are also known to cause bone and joint infections. *Propionibacterium acnes* is reported in approximately 10% of Prosthetic Joint Infections, but its frequency is likely underestimated due to the short incubation times of primary cultures in many clinical laboratories and its growth being considered as contamination. While fusidic acid is known to be active against staphylococci and has been reported to be active against *P. acnes*, this study was conducted to determine the susceptibility of contemporary *P. acnes* strains isolated from wounds and bone and joint infections. The determination of susceptibility was especially important because of the topical use of fusidic acid and the potential for fusidic acid to select for resistance.

Methods. MICs of fusidic acid and comparator drugs were determined for 51 clinical isolates of *P. acnes* cultured from normally sterile body sites (e.g., bone, blood, joints) of patients at the University of Rochester Medical Center, Rochester, NY, in calendar years 2011 and 2012. MICs were determined by broth microdilution in Brucella broth supplemented with hemin (5 µg/ml), vitamin K1 (1 µg/ml) and lysed horse blood (5%) with anaerobic incubation at 37°C for 48 h as recommended by CLSI M11-A7.

Results. The range of MICs and MIC₉₀s for fusidic acid and comparator drugs were determined. Fusidic acid MIC range was 0.03-1 µg/ml with a MIC₉₀ of 1 µg/ml. Among other antibiotics used for bone and joint infections, vancomycin had an MIC range of 0.12-1 µg/ml and the MIC₉₀ was 1 µg/ml, while daptomycin was less active with an MIC range of 0.5-8 µg/ml and an MIC₉₀ of 4 µg/ml. Among oral antibiotics, linezolid had an MIC range of ≤0.03-0.5 and an MIC₉₀ of 0.5 µg/ml. Levofloxacin had an MIC range of 0.06-2 µg/ml and an MIC₉₀ of 1 µg/ml.

Conclusion. In conclusion, fusidic acid had potent activity against *P. acnes*. The MIC of fusidic acid for all strains was ≤1 µg/ml. Fusidic acid could provide coverage in bone and joint infections for gram positive bacteria other than staphylococci, such as *P. acnes*.

Introduction

Propionibacterium acnes is often found in bone and joint infections, either in combination with staphylococci or as a single pathogen (1). Repeated culture is often necessary to rule out the chance that it is a contaminant. *P. acnes* is an anaerobic-aerotolerant Gram-positive bacillus that resides in sebaceous gland and hair follicles of the skin (1). *P. acnes* can form biofilm in bone and joint infections.

Penicillin G and ceftriaxone are considered antibiotics of first choice, with vancomycin and daptomycin as alternatives in case of β-lactam allergy or antimicrobial resistance. Clindamycin, tetracycline, and levofloxacin are oral alternatives. Rifampin is often used to overcome biofilm penetration and is considered active against *P. acnes*. Although considered an anaerobe, *P. acnes* is intrinsically resistant to metronidazole. Aminoglycosides generally have weak activity against *P. acnes*.

Fusidic acid (sodium fusidate, CEM-102) is in development for bone and joint infections, including prosthetic joint infections. The in vitro activity of fusidic acid against *Propionibacterium* species, including *P. acnes* was reported in the 1970's (2). This study examined the susceptibility of contemporary strains of *P. acnes* to fusidic acid.

Materials and Methods

Drugs. MICs of the following drugs were determined: azithromycin (USP, Lot# G), cefdinir (Sigma, Lot# 117K1392), cefixime (USP, Lot# F), cefpodoxime (USP, Lot# HDK009), clarithromycin (USP, Lot# GIG324), daptomycin (Sigma, Lot# SLBD03050V), doxycycline (Sigma, Lot# BCBF9827V), sodium fusidate (Cempra, Lot# WH-52-200-24), levofloxacin (Sigma, Lot# BCBF7004V), linezolid (Sigma, Lot# 020M4707V), penicillin (Sigma, Lot# BCBF3866V), trimethoprim/sulfamethoxazole (Sigma, Lot# O00M4110V and BCBF0534V), and vancomycin (Sigma, Lot# 120M1495V). Drugs were dissolved and diluted for testing per recommendations in CLSI M100-S22. Stock solutions of sodium fusidate were prepared on each day of testing.

Organisms. MICs of sodium fusidate and comparator drugs were determined for 51 clinical strains of *P. acnes* cultured from normally sterile body sites (e.g., bone, blood, joints) of patients at the University of Rochester Medical Center, Rochester, NY, in calendar years 2011 and 2012.

MIC Determinations. MICs of sodium fusidate and comparator drugs were determined by broth microdilution methodology in Brucella broth supplemented with hemin (5 µg/ml), vitamin K1 (1 µg/ml) and lysed horse blood (5%) as recommended by CLSI M11-A7 (3). Prior to testing, organisms were subcultured onto Brucella Blood Agar supplemented with hemin and vitamin K1 agar for 48 hours at 37°C in an anaerobic environment. Organism suspensions harvested from fresh agar cultures were adjusted to yield a final test concentration of 1 x 10⁶ CFU/mL. Microdilution trays containing diluted drugs and inoculated with test organisms were incubated at 37°C in an anaerobic environment for 48 hours. The MIC endpoint for a drug was read as the concentration at which no growth, or a significant reduction of growth, was observed after incubation.

The performance of test reagents (including drug potency), equipment, and test personnel were monitored using anaerobic quality control organisms as recommended by CLSI M100-S22 (4). Monitoring of drug potency using aerobic quality control organisms as recommended by CLSI was performed for those drugs lacking CLSI-approved anaerobic quality control ranges. MICs of all drugs for quality control organisms tested in parallel with test organisms were within acceptable ranges as recommended by CLSI.

Results

MICs of fusidic acid and comparator drugs for 51 clinical strains of *P. acnes* were determined. The range of MICs, MICs for 50% of strains and MICs for 90% of strains for all drugs are presented in Table 1. The frequency distributions of MICs of each drug for all strains tested are presented in Table 2.

Table 1. MICs of Fusidic Acid and Comparator Drugs against *P. acnes* (n=51)

Drugs	MIC (µg/mL)		
	MIC Range	MIC 50%	MIC 90%
Fusidic acid	0.03 - 1	0.5	1
Penicillin	≤0.03 - 1	≤0.03	0.06
Cefdinir	≤0.015 - 0.12	0.03	0.12
Cefixime	≤0.03 - 0.5	0.25	0.5
Cefpodoxime	≤0.015 - 2	0.5	2
Vancomycin	0.12 - 1	1	1
Clarithromycin	≤0.015 - 0.5	≤0.015	0.25
Azithromycin	≤0.015 - >32	0.06	4
Daptomycin	0.5 - 8	2	4
Doxycycline	≤0.008 - 1	0.06	0.12
Levofloxacin	0.06 - 2	1	1
Linezolid	≤0.03 - 0.5	0.25	0.5
Trimethoprim/ Sulfamethoxazole	0.06/1.19 - 2/38	0.25/4.75	0.5/9.5

Results (continued)

Table 2. Distribution MICs of Fusidic Acid and Comparator Drugs against *P. acnes* (n=51)

Drugs	MIC (µg/mL)													
	≤0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	>32
Fusidic acid			2	2	1	1	20	25						
Penicillin			35	15				1						
Cefdinir	13	15	9	14										
Cefixime		3	4	16	11	17								
Cefpodoxime	1		5	6	7	9	11	12						
Vancomycin					1	18	6	26						
Clarithromycin	40	3	1		5	2								
Azithromycin	2	6	19	16						3	2	1	1	1
Daptomycin							1	2	36	11	1			
Doxycycline	3	5	14	8	18	1	1	1						
Levofloxacin				1	5	13	5	24	3					
Linezolid			1		6	29	15							
Trimethoprim/ Sulfamethoxazole					1	14	12	22	1	1				

Conclusions

The MIC of sodium fusidate for 100% of the 51 clinical strains of *P. acnes* tested was ≤1 µg/mL. MICs of sodium fusidate were also generally uniform in distribution; the MIC of fusidic acid for 88% of test organisms ranged from 0.5-1 µg/mL. Of the drugs tested, penicillin is the only drug with CLSI-approved interpretive criteria for anaerobic organisms. Of the 51 strains of *P. acnes* tested, 98% and 2% of isolates, respectively, were susceptible or intermediate to penicillin. Although several antibiotics demonstrate in vitro activity they may not be available for oral long term use or may not be able to penetrate biofilm.

Fusidic acid is known to be effective in bone and joint infections ex-US and has been used orally for chronic long term suppression of bone and joint infections. Although in most clinical specimens *P. acnes* may be considered a contaminant, in bone and joint infections it is considered as a low virulence pathogen, especially when isolated multiple times. Contrary to a previous publication (2), all of the US *P. acnes* strains tested were susceptible to fusidic acid at MIC < 1 µg/mL. This difference could be related in part to the fact that fusidic acid is not approved for use in the US, whereas it has been used for several decades overseas, especially topically. Topical use has been associated with resistance development in staphylococci (5).

In conclusion, fusidic acid shows potential for use against *P. acnes* in addition to staphylococci in bone and joint infections.

References

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