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Fusidic Acid Activity and Coverage of Gram-positive Pathogens Associated with Acute Bacterial Skin and Skin Structure Infections (ABSSSI) in the USA (2008-2010)

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Abstract

Objectives: To determine the susceptibility (S) rates and activity of fusidic acid (FA; CEM-102) tested against Gram-positive pathogens that cause ABSSSI, isolated in the USA during 2008-2010 (16,033 strains) using CLSI reference broth microdilution methods and the EUCAST (≤1 mg/L) S breakpoint concentration.

Methods: *Staphylococcus aureus* (SA; 12,061 strains, 52% MRSA), coagulase-negative staphylococci (CoNS; 2,062 strains, 71% methicillin-resistant [R]), and 1,910 beta-haemolytic streptococci (BHS; 684 group A and 933 group B). Totals of 4940, 5006 and 6087 strains were tested in 2008, 2009 and 2010, respectively from 65 medical centers in 37 states including all nine Census Regions. Organisms were predominantly from bacteremias (61%), ABSSSI (25%) and pneumonia (14%), and tested by the CLSI M07-A8 method. Non-S SA strains were tested by molecular methods to detect R mechanisms and by PFGE to determine possible clonality.

Results: FA was consistently active against SA (MIC_{90}, 0.12 mg/L) across all years (2008-2010) without significant change in the S rate (99.74% at ≤1 mg/L). MRSA and methicillin-S SA had nearly the same FA-S rates and $MIC_{50/90}$ results, but MR-CoNS were slightly less S (90.76%) than MS-CoNS (97.28%) strains. BHS were less inhibited by FA ($MIC_{50/90}$, 8/16 mg/L), however 99.42% of Group A (*S. pyogenes*) isolates were inhibited at ≤8 mg/L (FA PK trough concentration = 80 mg/L). 31 SA strains had MIC values at 2->8 mg/L with leading R mechanisms detected of *fusA* (7; M453I, L461S, A471V + P404L [2], A477 deletion, L641K [2], V92A), *fusB* (4) *fusC* (17) and *fusE* (2; G78 to Q99 deletion). R-mechanisms were found among all tested strains with FA MIC at 2 mg/L or greater. Clonal occurrences were noted within or between monitored years in 3 hospitals; 3 states (New York, Michigan, Oregon).

Pathogen (no. tested)	Fusidic acid MIC (mg/L)		
	50%	90%	%S (% at ≤8 mg/L)
SA (12,061)	0.12	0.12	99.74 (99.98)
MRSA (6,245)	0.12	0.12	99.81 (99.97)
MSSA (5,816)	0.12	0.12	99.67 (100.0)
CoNS (2,062)	0.12	0.25	92.62 (98.64)
MR (1,473)	0.12	0.25	90.76 (98.37)
MS (589)	0.12	0.25	97.28 (99.32)
BHS (1,910)	8	16	0.26 (85.97)
Group A (684)	4	8	0.44 (99.42)

Conclusions: FA remains highly active against SA (99.74% S) and other ABSSSI pathogens isolated in the USA. CoNS were slightly less S at ≤1 mg/L (92.62%) and 99.42% of *S. pyogenes* were inhibited at ≤8 mg/L. FA-R mechanisms were dominantly acquired (67.7% *fusB* or *C*). FA appears to be an excellent, orally-administered (with a novel loading-dose strategy), systemic drug candidate against the relatively naïve staphylococcal population in the USA.

Introduction

Fusidic acid, also known as CEM-102 (Cempra Pharmaceuticals, Chapel Hill, North Carolina, USA), is a steroidal class antimicrobial agent initially identified from *Fusidium coccineum* by Godtfredsen et al. in 1960. Such steroidal agents, however, have no corticosteroid activity, yet exhibit a well characterized potency against staphylococci, including methicillin-resistant *Staphylococcus aureus* (MRSA) and coagulase-negative staphylococcal species (CoNS). Fusidic acid was introduced into clinical trials in 1962 as a potential systemic and topical therapy for staphylococcal skin and skin structure infections.

The structure of fusidic acid as a sodium salt is water soluble and active via the oral route (molecular weight, 538.7; pK at 5.35). Biedenbach and colleagues recently redefined the fusidic acid spectrum and activity against a wide range of pathogens as follows: *S. aureus* (MIC, 0.25 mg/L), *Micrococcus luteus* (MIC, 0.25-0.5 mg/L), *Corynebacterium* spp. (MIC, 0.06-0.12 mg/L), *Moraxella catarrhalis* (MIC, 0.06-0.12 mg/L) and *Neisseria meningitidis* (MIC, 0.12-0.25 mg/L); *Streptococcus* spp., not *S. pyogenes* (MIC, 16-32 mg/L), and enterococci (MIC, 2-8 mg/L) were less susceptible with Gram-negative bacilli being frankly resistant at fusidic acid MIC values of ≥32 mg/L. This range of activity is the result of drug interactions with elongation factor G (EF-G) that prevents its release from the ribosome, thus compromising protein synthesis; a mode of action that continues to be actively studied.

Fusidic acid resistances have long been thought to be caused by mutations of the EF-G-encoding gene. More recently acquired mechanisms (*fus*B and C) were detected as mobile elements that can either be chromosomal- or plasmid-mediated in staphylococci. At least five mechanisms exist (*fus*A-E), producing staphylococcal resistances correlating with fusidic acid MIC values at ≥2 mg/L. As this antimicrobial was used clinically worldwide, microbiologists in some nations (Europe and Australia) encountered Gram-positive pathogens with elevated fusidic acid resistance rates; in contrast, in the United States (USA) the Food and Drug Administration (FDA) has not approved this agent for therapeutic use by any route of administration. Thus, this unique steroidal antimicrobial, if used in the USA would be prescribed for treatment of *Staphylococcus* species.

In this in vitro report, we summarize the results of a fusidic acid resistance surveillance study in the USA for 2008-2010. We also describe investigations defining the molecular basis of fusidic acid resistance rates among *S. aureus*.

Materials and Methods

Bacterial strains. A total of 12,061 *S. aureus* strains collected during 2008-2010 (4,940 to 6,086 isolates/year) in 65 USA hospitals, located in the nine Census Regions (37 states) were analyzed as part of the SENTRY Antimicrobial Surveillance Program. These isolates were obtained from bloodstream (BSI; 60.7%), respiratory tract infections (14.2%), and skin and skin structure infections (ABSSSI; 25.1%), according to defined protocols. Also 3,972 other Gram-positive organisms were sampled as follows: CoNS (2,062), and β-haemolytic streptococci (1,910; 684 *S. pyogenes*). Only one isolate per patient from documented infections were included. Species identification was confirmed by standard biochemical tests, the Vitek 2 System (bioMerieux, Hazelwood, Missouri, USA) or 16S rRNA sequencing, when necessary.

Antimicrobial susceptibility testing. Isolates were susceptibility tested by a reference broth microdilution procedure as described by the Clinical and Laboratory Standards Institute (CLSI) using validated broth microdilution panels manufactured by TREK Diagnostics (Cleveland, Ohio, USA). Categorical interpretations for all antimicrobials were those found in M100-S21 (2011) and quality control (QC) was performed using, *S. aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212 and *S. pneumoniae* ATCC 49619. All QC results were within specified ranges as published in CLSI documents. For fusidic acid, the interpretive susceptibility criteria of the EUCAST group (2011) were applied at ≤1 mg/L; and QC ranges were used based on the recent study reported by Jones and Ross (2009).

Detection of fusidic acid resistance mechanisms. All strains displaying fusidic acid MIC at ≥2 mg/L (EUCAST non-susceptible breakpoint) were tested for the presence of *fusB*, *fusC* and *fusD* in a multiplex PCR approach. Detection of *fusD* (intrinsic in *S. saprophyticus*) was included in this reaction to detect strains that were incorrectly identified as other staphylococcal species.

Constitutive genes *fusA* and *fusE* were amplified and sequenced using Extensor Hi-fidelity Master Mix (ABGene, Sussex, United Kingdom) as well as custom and previously described oligonucleotides. Sequencing was performed in five and two reactions, respectively. The nucleotide sequences and deduced aminoacid sequences were analyzed using the Lasergene software package (DNASTAR, Madison, Wisconsin, USA) and compared with sequences available through the internet using BLAST (http://www.ncbi.nlm.nih.gov/blast/).

Results

- Three years of fusidic acid antimicrobial surveillance has been completed in the USA (16,033 Gram-positive pathogen isolates) using the CLSI reference methods and interpretive breakpoints used by EUCAST.
- Fusidic acid ($MIC_{50/90}$, 0.12 mg/L) inhibited 99.7% of *S. aureus* sampled at ≤1 mg/L, and MRSA strains (99.9%) were slightly more susceptible than MSSA strains (99.7%, see Table 1).
- Other orally-administered agents active against MRSA (% susceptible) were: doxycycline (96.0%), linezolid (99.9%) and TMP/SMX (98.2%). Parenteral and topical antimicrobials such as vancomycin (≥99.9%) and mupirocin (96.5%) were also active (Table 2).
- Fusidic acid ($MIC_{50/90}$, 0.12/0.25 mg/L), like comparison agents, was less active against CoNS strains (92.5% susceptible, Tables 1 and 2).
- BHS strains were less susceptible to fusidic acid (MIC_{50}, 8 mg/L); but *S. pyogenes* was most inhibited (MIC_{90}, 8 mg/L) by fusidic acid (99.4% of isolates inhibited at ≤8 mg/L; Table 2).
- Molecular characterization of all 31 fusidic acid-resistant (MIC, ≥2 mg/L) *S. aureus* strains revealed diverse mechanisms (Table 3). These strains were isolated from 15 states, mostly from New York (4 isolates). Acquired *fusB* (4) and *C* (17) genes predominated (21/31, 67.7%), and *fusA* (8) and *E* (2) target mutations were more rare. The *fusA* mutations were varied, and occurred in five states.
- Clonal occurrences were noted in *fusA* and *fusE* strains from Oregon and Michigan, respectively.

Table 2. Fusidic acid activity compared to other classes of antimicrobials used for oral therapy of ABSSSI. USA isolates (2008-2010) of MRSA, MR-CoNS and *S. pyogenes* (8,401 strains).

Organism (no. tested) Antimicrobial agent	MIC (mg/L) 50%	MIC (mg/L) 90%	% susceptible[a] CLSI/EUCAST
MRSA (6,245)			
Fusidic acid	0.12	0.12	-/99.8
Ciprofloxacin	>4	>4	27.2/27.2
Clindamycin	≤0.25	>2	66.6/66.1
Doxycycline	≤0.12	0.5	98.4/96.0
Erythromycin	>2	>2	8.3/8.3
Levofloxacin	4	>4	28.8/28.8
Linezolid	1	2	99.9/99.9
TMP/SMX[b]	≤0.5	≤0.5	98.2/98.2
Mupirocin	≤4	≤4	(96.5)[c]
Vancomycin[e]	1	1	>99.9/>99.9
MR-CoNS (1,473)			
Fusidic acid	0.12	0.25	-/90.7
Ciprofloxacin	>4	>4	32.4/32.4
Clindamycin	≤0.25	>2	59.1/57.2
Doxycycline	0.5	2	94.1/85.9
Erythromycin	>2	>2	23.7/24.0
Levofloxacin	>4	>4	32.4/32.4
Linezolid	0.5	1	97.8/97.8
TMP/SMX[b]	2	>2	52.0/52.0
Mupirocin	16	>256	(61.3)[c]
Vancomycin[e]	2	2	100.0/99.3
S. pyogenes (684)			
Fusidic acid	4	8	-/- (99.4)[d]
Ciprofloxacin	0.5	1	92.1/92.1
Clindamycin	≤0.25	≤0.25	96.4/96.4
Doxycycline	≤0.12	8	86.8/85.9
Erythromycin	≤0.25	1	87.6/87.6
Levofloxacin	≤0.5	1	99.4/93.4
Linezolid	1	1	100.0/100.0
Penicillin	≤0.03	≤0.03	100.0/100.0
TMP/SMX[b]	≤0.5	≤0.5	-/-
Vancomycin[e]	0.25	0.5	100.0/100.0

a. Susceptible breakpoints of the CLSI/EUCAST (2011).
b. TMP/SMX = trimethoprim/sulfamethoxazole (1:19 ratio, TMP concentration shown).
c. Topical agent using a high-level resistance breakpoint of ≤256 mg/L (for comparison only).
d. %inhibited at ≤8 mg/L in parentheses.
e. Vancomycin results show high in vitro efficacy of a commonly used parenteral agent.

Table 3. Fusidic acid resistance mechanisms among *S. aureus* strains (MIC, ≥2 mg/L) collected from USA medical centers 2008-2010.

Study year (no. of strains)	Location (no. of strains)	Acquired genes (No.) *fusB*	Acquired genes (No.) *fusC*	Mutations (No.) *fusA*	Mutations (No.) *fusE*
2008 (11)	Arkansas (1)	-	1	-	-
	Hawaii (3)	-	3	-	-
	Iowa (2)	-	2	-	-
	Kentucky (1)	-	-	M453I (1)	-
	Michigan (3)	-	1	-	78G to Q99 deletion (2)[a]
	Ohio (1)	1	-	-	-
2009 (10)	California (2)	-	2	-	-
	Massachusetts (1)	1	-	L461S (1)	-
	Michigan (1)	-	-	-	-
	New Jersey (1)	-	1	-	-
	New York (1)	-	1	-	-
	Ohio (1)	-	1	-	-
	Oregon (2)	-	-	A71V, P404L (2)[a]	-
	Tennessee (1)	1	-	-	-
2010 (10)	Massachusetts (3)	-	1	L461K (2)	-
	Missouri (1)	1	-	-	-
	North Carolina (1)	-	-	V92A (1)	-
	New York (4)	-	4	-	-
	Utah (1)	-	-	A477 deletion (1)	-
All years (31)	15 states	4	17	8	2

a. Clonal occurrence.

Table 1. Fusidic acid MIC distributions for staphylococcal and *Streptococcus* spp. associated with ABSSSI isolated in USA medical centers in 2008-2010 (16,033 strains).

Organism/subsets (no. tested)[a]	Occurrences (cum. %) at MIC in mg/L ≤0.06	0.12	0.25	0.5	1	2	4	8	MIC (mg/L) 50%	MIC (mg/L) 90%	% susceptible[b]
S. aureus											
All (12,061)	2,687 (22.3)	8,271 (90.8)	963 (98.8)	88 (99.6)	21 (99.7)	7 (99.8)	14 (99.9)	8 (>99.9)[c]	0.12	0.12	99.74
MSSA (5,816)	1,368 (23.5)	3,924 (91.0)	461 (98.9)	39 (99.6)	5 (99.7)	3 (99.7)	9 (99.9)	7 (100.0)	0.12	0.12	99.67
MRSA (6,245)	1,319 (21.1)	4,347 (90.7)	502 (98.8)	49 (99.6)	16 (99.8)	4 (99.9)	5 (>99.9)	1 (>99.9)	0.12	0.12	99.81
CoNS											
All (2,062)	455 (22.1)	1,282 (84.3)	155 (91.8)	11 (92.3)	6 (92.6)	15 (93.3)	30 (94.8)	80 (98.6)[d]	0.12	0.25	92.58
MS (589)	153 (26.0)	372 (89.1)	39 (95.8)	6 (96.8)	3 (97.3)	3 (97.8)	3 (98.3)	6 (99.3)	0.12	0.25	97.28
MR (1,473)	302 (20.5)	910 (82.3)	116 (90.2)	5 (90.5)	3 (90.7)	12 (91.5)	27 (93.4)	74 (98.4)	0.12	0.25	90.70
BHS											
All (1,910)	-	1 (<0.1)	0 (0.1)	1 (0.1)	3 (0.3)	53 (3.0)	701 (39.7)	883 (86.0)	8	>8	0.26 (85.97)[e]
S. pyogenes (684)	-	1 (0.2)	0 (0.2)	0 (0.2)	2 (0.4)	27 (4.4)	486 (75.4)	164 (99.4)	4	8	0.44 (99.42)[e]
Others (1,226)	-	-	-	1 (0.1)	1 (0.2)	26 (2.3)	215 (19.8)	719 (78.5)	8	>8	0.16 (78.47)[e]

a. MSSA = methicillin-susceptible *S. aureus*, MRSA = methicillin-resistant *S. aureus*, MS = methicillin-susceptible, and MR = methicillin-resistant.
b. Susceptibility breakpoints of EUCAST (2011 at ≤1 mg/L).
c. Two strains at >8 mg/L.
d. 28 strains at >8 mg/L.
e. % of BHS with MIC at ≤8 mg/L.

Conclusions

- Fusidic acid remains an excellent candidate for ABSSSI treatment in the USA, covering 99.74% of *S. aureus* strains at ≤1 mg/L. Other cutaneous pathogens (CoNS and *S. pyogenes*) were also inhibited at achievable drug levels.
- Among the 31 fusidic acid-resistant *S. aureus* detected in 2008-2010 (12,061 total strains), *fusA* (8), *fusB* (4), *fusC* (17) and *fusE* (2) mechanisms were observed with acquired genes being most often found. Only two *fusA* mutants (L461K) demonstrated high level resistance (≥512 mg/L).
- Against the USA population of *S. aureus*, fusidic acid appears to be highly active (MIC_{90}, 0.12 mg/L; 0.26% non-susceptible), without negative influences of methicillin susceptibility patterns, or resistance selective pressure via prior clinical use (oral, parenteral or topical). Continued fusidic acid clinical trials are warranted for oral therapy of ABSSSI with the optimal dosing regimen that has been shown to minimize resistance development.

Selected References

1. Biedenbach DJ, Rhomberg PR, Mendes RE, Jones RN (2010). Spectrum of activity, mutation rates, synergistic interactions, and the effects of pH and serum proteins for fusidic acid (CEM-102). *Diagn Microbiol Infect Dis*. 66:301-307.
2. Castanheira M, Watters AA, Bell JM, Turnidge JD, Jones RN (2010). Fusidic acid resistance rates and prevalence of resistance mechanisms among *Staphylococcus* spp. isolated in North America and Australia, 2007-2008. *Antimicrob Agents Chemother*. 54:3614-3617.
3. Castanheira M, Watters AA, Mendes RE, Farrell DJ, Jones RN (2010). Occurrence and molecular characterization of fusidic acid resistance mechanisms among *Staphylococcus* spp. from European countries (2008). *J Antimicrob Chemother*. 65:1353-1358.
4. Clinical and Laboratory Standards Institute (2009). *M07-A8. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard: eighth edition.* Wayne, PA: CLSI.
5. Clinical and Laboratory Standards Institute (2011). *M100-S21. Performance standards for antimicrobial susceptibility testing: 21st informational supplement*. Wayne, PA: CLSI.
6. EUCAST (2011). Breakpoint tables for interpretation of MICs and zone diameters. Version 1.3, January 2011. Available at: http://www.eucast.org/clinical_breakpoints/. Accessed: March 18, 2011.
7. Jones RN, Ross JE (2009). Initial quality control ranges for CEM-102 (fusidic acid) using the CLSI multi-laboratory M23-A3 study design In: *49th ICAAC, September 12-15, 2009.* San Francisco, CA, USA.
8. Jones RN, Mendes RE, Sader HS, Castanheira M (2011). In vitro Antimicrobial Findings for Fusidic Acid Tested Against Contemporary (2008-2009) Gram-Positive Organisms Collected in the United States. *Clin Infect Dis.* (*In press*).
9. Pfaller MA, Castanheira M, Sader HS, Jones RN (2010). Evaluation of the activity of fusidic acid tested against contemporary Gram-positive clinical isolates from the USA and Canada. *Int J Antimicrob Agents* 35:282-287.
10. McLaws F, Chopra I, O'Neill AJ (2008). High prevalence of resistance to fusidic acid in clinical isolates of *Staphylococcus epidermidis*. *J Antimicrob Chemother*. 61:1040-1043.
11. McLaws F, Larsen AR, Skov RL, Chopra I, O'Neill AJ (2011). Distribution of fusidic acid determinants. *Antimicrob Agents Chemother*. 55:1177-1181.
12. Nagaev I, Bjorkman J, Andersson DI, Hughes D (2001). Biological cost and compensatory evolution in fusidic acid-resistant *Staphylococcus aureus*. *Mol Microbiol.* 40:433-439.
13. Tsuji BT, Okusanya OO, Bulitta JB, Forrest A, et al. (2011) Application of pharmacokinetic-pharmacodynamic modeling and the justification of a novel fusidic acid dose regimen: Raising Lazarus from the dead. *Clin Infect Dis.* (*In press*).