Activity of Fusidic Acid against Staphylococci Isolated from Patients in United States Hospitals during 2014

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AMENDED ABSTRACT

Background: Fusidic acid (FA) is an established anti-staphylococcal agent used in clinical practice in Europe, Australia and Canada for at least three decades. FA is currently under clinical development in the USA. This study assessed the activities of FA and comparators tested against *Staphylococcus aureus* (SA) and coagulase-negative staphylococci (CoNS) isolates. Mutation analysis was performed by PCR and sequencing.

Methods: SA (1,804 isolates) and CoNS (193 isolates) were collected in 2014 from 26 medical centers located in the USA. Identification was performed by standard algorithms. Isolates were tested for susceptibility (S) by CLSI methods (M07-A10 and M100-S25). Resistance mechanisms were detected by PCR (*fusB, fusC, fusD*) and sequencing (*fusA, fusE*). PFGE was performed to determine clonality.

Results: FA ($MIC_{50/90}$, 0.12/0.12 µg/mL) inhibited 99.8% (1800/1804) of the SA strains at ≤1 µg/mL. Using EUCAST breakpoints, FA susceptibility rates were very high, regardless of the methicillin-susceptible/resistant profile (99.6% for MSSA and 100.0% for MRSA). Three SA strains, isolated from patients in Iowa, New York, and Florida were positive for *fusc and* had FA MICs of 4 to 8 µg/mL. One strain, from a patient from Georgia, had a L461K substitution in *fusA* and a FA MIC of >16 µg/mL. A total of 179 of the 193 (92.7%) CoNS strains were inhibited by FA at MIC values ≤1 µg/mL. The activity of FA against CoNS demonstrated differences between the MS/MR subsets. MS-CoNS displayed MIC_{90} results of 0.12 µg/mL with all isolates inhibited at MIC values ≤0.25 µg/mL, whereas the MIC_{90} was 2 µg/mL for MR-CoNS (representing 69.9% of strains). FA resistance mechanisms found in CoNS were (n): *fusB* (9), *fusC* (3), and D597E substitution in *fusA* (1). PFGE revealed that none of CoNS strains were clonally related – including strains found in the same medical center.

Conclusions: Compared to previous surveys, FA demonstrated sustained and potent activity against this current collection of staphylococci from USA hospitals. A variety of FA resistance mechanisms were found and epidemiology (demographics and PFGE) did not reveal any evidence of clonality.

INTRODUCTION

Sodium fusidate (CEM-102), the sodium salt of fusidic acid, is a steroidal antibiotic initially isolated from *Fusidium coccineum* in 1960. Such steroidal agents, however, have no corticosteroid activity, yet possess a well characterized potency against Gram-positive bacteria such as staphylococci, including methicillin-resistant *Staphylococcus aureus* (MRSA) and coagulase-negative staphylococcal species (CoNS). Fusidic acid was introduced into clinical trials in Europe in 1962 as a potential systemic and topical therapy for staphylococcal skin and skin structure infections and has also been used for long-term treatment of bone and joint infections.

Fusidic acid spectrum of activity has been defined against a wide range of pathogens which includes *S. aureus* (MIC_{90}, 0.25 µg/mL), *Corynebacterium* spp. (MICs, 0.06-0.12 µg/mL), and *Propionibacterium acnes* (MICs, 0.03-1 µg/mL). *Streptococcus* spp., including *S. pyogenes* (MICs, 2-8 µg/mL), and enterococci (MICs, 2-8 µg/mL), had higher MIC's *in vitro*, and Gram-negative bacilli were frankly resistant to fusidic acid with MIC values ≥32 µg/mL. This range of activity is the result of interactions with elongation factor G (EF-G) that prevents the release of the newly formed peptide amino acid chain from the ribosome, thus compromising protein synthesis, a mode of action that continues to be actively studied.

Resistance to fusidic acid has long been thought to be caused by mutations of the EF-G-encoding gene (*fus*A). 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 currently exist (*fus*A-E). With years of worldwide clinical use, microbiologists in some nations (e.g., United Kingdom, Ireland, Greece) have encountered Gram-positive pathogens with increased fusidic acid resistance rates. In contrast, in the United States (USA), where fusidic acid is not approved for therapeutic use by any route of administration by the USA Food and Drug Administration (FDA), resistance rates remain very low. Thus, this unique antimicrobial, if used in the USA, would be prescribed for treatment of a naïve population of Gram-positive bacteria, including *S. aureus*.

In the present study, we examined the activity of fusidic acid and comparator agents against 1,997 contemporary clinical bacterial strains collected in 2014 from respiratory tract infections (RTI), acute bacterial skin and skin structure infections (ABSSSI), and bloodstream infections (BSI) in USA medical centers.

METHODS

Organisms and sources: A total of 1,997 non-duplicated staphylococcal strains were collected prospectively from 26 medical centers located in the USA. These strains were recovered consecutively from patients with ABSSSI, BSI, respiratory tract infections, and fewer numbers of strains from other sources of infection.

Strains were identified by the submitting laboratories and confirmed by JMI Laboratories (North Liberty, Iowa, USA) using standard bacteriologic algorithms and methodologies, including the use of Vitek Identification Systems (bioMerieux, Hazelwood, Missouri, USA), matrix-assisted laser desorption ionization – time of flight (MALDI-TOF) mass spectrometry (Bruker Daltonics, Billerica, Massachusetts, USA), and 16S rRNA sequencing.

Susceptibility test methods: CLSI M07-A10 (2015) using validated broth microdilution trays produced by Thermo Fisher Scientific Inc., formerly TREK Diagnostics (Cleveland, Ohio, USA) were tested in cation-adjusted Mueller-Hinton broth. Interpretive criteria were those of the CLSI (M100-S25; 2015) and EUCAST (2015).

Fusidic acid staphylococcal breakpoints were applied for comparison purposes only, according to EUCAST criteria (≤1 µg/mL).

Quality control (QC) per the CLSI M07-A10 (2015), and CLSI M100-S25 (2015) recommendations and guidelines using the following strains, as appropriate: *S. aureus* ATCC 29213 and *S. pneumoniae* ATCC 49619. All QC results were within CLSI published ranges for fusidic acid. Comparison antimicrobial agents were within the ranges as published in CLSI M100-S25 (data on file at JMI Laboratories).

Molecular methods: Resistance mechanisms were detected by PCR (*fusB, fusC, fusD*) and sequencing (*fusA, fusE*). PFGE was performed to determine potential clonality.

RESULTS

- The spectrum of activity and potencies of fusidic acid and comparator agents tested against staphylococcal clinical strains from this 2014 surveillance program are presented in **Tables 1** and **2**. A total of 1,997 isolates were evaluated and MIC distributions by species, group, and methicillin susceptible/resistance phenotype are shown in **Table 1**.
- For *S. aureus,* fusidic acid ($MIC_{50/90}$, 0.12/0.12 µg/mL) inhibited 99.8% (1800/1804) of isolates at ≤1 µg/mL. Using EUCAST breakpoints, fusidic acid susceptibility rates were very high among USA strains, regardless of the methicillin-susceptible/resistant profile (99.6% for methicillin-susceptible *S. aureus* (MSSA) and 100.0% for MRSA). Among comparator agents with available oral formulations, linezolid, clindamycin, tetracycline and trimethoprim-sulfamethoxazole (TMP-SMX) demonstrated high susceptibility rates against *S. aureus* strains at 99.9, 83.5, 94.9 and 97.9%, respectively (CLSI). Erythromycin and levofloxacin resistance rates were high at 53.9 and 36.0%, respectively (**Table 2**). Overall against *S. aureus*, susceptibility rates were highest for agents administered by the parenteral route: vancomycin (100.0%), gentamicin (97.7%) and daptomycin (99.9%).
- Four *S. aureus* strains from USA medical centers displayed fusidic acid values of >2 µg/mL. Three, from patients in Iowa, New York, and Florida, were positive for *fusC* and had MIC values from 4 to 8 µg/mL. One isolate, from a patient in Georgia, had a L461K substitution in *fusA* and an MIC >16 µg/mL.
- For CoNS, a total of 179 of the 193 (92.7%) strains were inhibited by fusidic acid at MIC values at ≤1 µg/mL. The activity of fusidic acid against CoNS demonstrated differences between the methicillin-susceptible (MS)/methicillin-resistant (MR) subsets. MSCoNS displayed MIC_{90} results of 0.12 µg/mL with all isolates inhibited at MIC values of ≤0.25 µg/mL, whereas for MRCoNS, the MIC_{90} was 2 µg/mL. MRCoNS represented 69.9% of all CoNS tested.
- Linezolid was the only comparator oral agent with wide coverage for CoNS isolates, inhibiting 99.0% of the strains at the current CLSI breakpoint. Clindamycin, TMP-SMX, and tetracycline demonstrated modest activity against these pathogens (69.4, 76.2, and 83.9% susceptible, respectively, CLSI). Erythromycin and levofloxacin susceptibility rates were 41.5 and 59.6%, respectively. All isolates were susceptible to vancomycin and daptomycin while gentamicin resistance was 16.6% (**Table 2**).
- Fusidic acid resistance mechanisms found in CoNS were (n): *fusB* (9), *fusC* (3), and a D597E substitution in *fusA* (1). PFGE analyses showed that none of the CoNS strains were clonally related, including strains found in the same medical center.

Table 1. Frequency distributions of fusidic acid when tested against *Staphylococcus* spp. recovered as part of the USA surveillance program for 2014.

Organism	No. of Isolates	No. of isolates (cumulative %) inhibited at MIC (µg/mL): ≤0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	>16	MIC_{50}	MIC_{90}
Staphylococcus aureus	1804	1 (0.1)	42 (2.4)	773 (45.2)	946 (97.7)	31 (99.4)	4 (99.6)	3 (99.8)	0 (99.8)	2 (99.9)	1 (>99.9)	0 (>99.9)	1 (100.0)	0.12	0.12
MSSA	956	1 (0.1)	23 (2.5)	397 (44.0)	515 (97.9)	14 (99.4)	2 (99.6)	0 (99.6)	0 (99.6)	2 (99.8)	1 (99.9)	0 (99.9)	1 (100.0)	0.12	0.12
MRSA	848	-[a]	19 (2.2)	376 (46.6)	431 (97.4)	17 (99.4)	2 (99.6)	3 (100.0)						0.12	0.12
Coagulase-negative staphylococci	193	-	11 (5.7)	101 (58.0)	65 (91.7)	2 (92.7)	0 (92.7)	0 (92.7)	2 (93.8)	4 (95.9)	8 (100.0)			0.06	0.12
MSCoNS	58	-	2 (3.4)	33 (60.3)	22 (98.3)	1 (100.0)								0.06	0.12
MRCoNS	135	-	9 (6.7)	68 (57.0)	43 (88.9)	1 (89.6)	0 (89.6)	0 (89.6)	2 (91.1)	4 (94.1)	8 (100.0)			0.06	2

a. "-" represents no isolates found at this MIC dilution.

Table 2. Activity of fusidic acid and comparator antimicrobial agents when tested against 1,997 isolates of *Staphylococcus* spp. (USA; 2014).

Antimicrobial agent (no. tested)	MIC (µg/mL) MIC_{50}	MIC_{90}	Range	CLSI[a] %S / %I / %R	EUCAST[a] %S / %I / %R
S. aureus (1,804)					
Fusidic Acid	0.12	0.12	≤0.015 – >16	-[b] / - / -	99.8 / - / 0.2
Erythromycin	16	>16	≤0.12 – >16	40.2 / 5.9 / 53.9	40.4 / 1.5 / 58.1
Clindamycin	≤0.25	>2	≤0.25 – >2	83.5 / 0.2 / 16.3	83.3 / 0.2 / 16.5
Vancomycin	1	1	0.25 – 2	100.0 / 0.0 / 0.0	100.0 / - / 0.0
Linezolid	1	1	0.25 – >8	99.9 / - / 0.1	99.9 / - / 0.1
Oxacillin	1	>2	≤0.25 – >2	53.0 / - / 47.0	53.0 / - / 47.0
Tetracycline	≤0.5	≤0.5	≤0.5 – >8	94.9 / 0.3 / 4.8	92.3 / 1.5 / 6.2
Gentamicin	≤1	≤1	≤1 – >8	97.7 / 0.2 / 2.1	97.6 / - / 2.4
Levofloxacin	0.25	>4	≤0.12 – >4	63.4 / 0.6 / 36.0	63.4 / 0.6 / 36.0
TMP-SMX[c]	≤0.5	≤0.5	≤0.5 – >4	97.9 / - / 2.1	97.9 / 0.2 / 1.9
Daptomycin	0.25	0.5	≤0.06 – 2	99.9 / - / -	99.9 / - / 0.1
MSSA (956)					
Fusidic Acid	0.12	0.12	≤0.015 – >16	- / - / -	99.6 / - / 0.4
Erythromycin	0.25	>16	≤0.12 – >16	64.4 / 7.5 / 28.1	64.7 / 2.3 / 33.0
Clindamycin	≤0.25	≤0.25	≤0.25 – >2	94.9 / 0.1 / 5.0	94.7 / 0.2 / 5.1
Vancomycin	1	1	0.5 – 2	100.0 / - / 0.0	100.0 / - / 0.0
Linezolid	1	1	0.25 – 2	100.0 / - / 0.0	100.0 / - / 0.0
Tetracycline	≤0.5	≤0.5	≤0.5 – >8	96.1 / 0.2 / 3.7	94.4 / 0.4 / 5.2
Gentamicin	≤1	≤1	≤1 – >8	99.2 / 0.0 / 0.8	99.0 / - / 1.0
Levofloxacin	0.25	2	≤0.12 – >4	90.0 / 0.4 / 9.6	90.0 / 0.4 / 9.6
TMP-SMX[c]	≤0.5	≤0.5	≤0.5 – >4	99.2 / - / 0.8	99.2 / 0.0 / 0.8
Daptomycin	0.25	0.5	≤0.06 – 1	100.0 / - / -	100.0 / - / 0.0
MRSA (848)					
Fusidic Acid	0.12	0.12	0.03 – 1	- / - / -	100.0 / - / 0.0
Erythromycin	>16	>16	≤0.12 – >16	12.9 / 4.1 / 83.0	13.1 / 0.5 / 86.4
Clindamycin	≤0.25	>2	≤0.25 – >2	70.6 / 0.4 / 29.0	70.5 / 0.1 / 29.4
Vancomycin	1	1	0.25 – 2	100.0 / 0.0 / 0.0	100.0 / - / 0.0
Linezolid	1	1	0.25 – >8	99.9 / - / 0.1	99.9 / - / 0.1
Tetracycline	≤0.5	1	≤0.5 – >8	93.6 / 0.5 / 5.9	90.0 / 2.8 / 7.2
Gentamicin	≤1	≤1	≤1 – >8	96.1 / 0.4 / 3.5	96.0 / - / 4.0
Levofloxacin	4	>4	≤0.12 – >4	33.5 / 0.7 / 65.8	33.5 / 0.7 / 65.8
TMP-SMX[c]	≤0.5	≤0.5	≤0.5 – >4	96.6 / - / 3.4	96.6 / 0.3 / 3.1
Daptomycin	0.25	0.5	≤0.06 – 2	99.8 / - / -	99.8 / - / 0.2
CoNS (193)[d]					
Fusidic Acid	0.06	0.12	0.03 – 8	- / - / -	92.7 / - / 7.3
Erythromycin	16	>16	≤0.12 – >16	41.5 / 2.0 / 56.5	42.5 / 1.0 / 56.5
Clindamycin	≤0.25	>2	≤0.25 – >2	69.4 / 2.6 / 28.0	66.3 / 3.1 / 30.6
Vancomycin	1	2	0.5 – 4	100.0 / 0.0 / 0.0	100.0 / - / 0.0
Linezolid	0.5	0.5	0.25 – >8	99.0 / - / 1.0	99.0 / - / 1.0
Oxacillin	1	>2	≤0.25 – >2	30.1 / - / 69.9	30.1 / - / 69.9
Tetracycline	≤0.5	>8	≤0.5 – >8	83.9 / 2.1 / 14.0	77.2 / 4.7 / 18.1
Gentamicin	≤1	>8	≤1 – >8	80.3 / 3.1 / 16.6	76.2 / - / 23.8
Levofloxacin	0.25	>4	≤0.12 – >4	59.6 / 0.0 / 40.4	59.6 / 0.0 / 40.4
TMP-SMX[c]	≤0.5	>4	≤0.5 – >4	76.2 / - / 23.8	76.2 / 13.0/ 10.9
Daptomycin	0.25	0.5	≤0.06 – 1	100.0 / - / -	100.0 / - / 0.0

a. Criteria as published by the CLSI [2015] and EUCAST [2015]. S = susceptible, I = intermediate and R = resistant.
b. "-" = no breakpoint defined.
c. TMP-SMX = Trimethoprim-sulfamethoxazole.
d. CoNS = coagulase-negative staphylococci; Includes: *Staphylococcus capitis* (12 strains), *S. caprae* (three strains), *S. cohnii* (one strain), *S. epidermidis* (108 strains), *S. haemolyticus* (14 strains), *S. hominis* (15 strains), *S. intermedius* (two strains), *S. lugdunensis* (25 strains), *S. pseudintermedius* (one strain), *S. simulans* (seven strains), *S. warneri* (four strains), and unspeciated *Staphylococcus* (one strain).

CONCLUSIONS

- Fusidic acid demonstrated sustained potent activity against *S. aureus*, including MRSA strains, isolated in the USA during 2014. Only four (0.2%) strains had increased fusidic acid MIC values (>2 µg/mL).
- Fusidic acid resistance among CoNS was 7.3% overall as per EUCAST criteria, and was found only in MRCoNS. No evidence of clonality was observed in the resistant strains.
- These results demonstrate that fusidic acid is a potentially valuable alternative for the treatment of staphylococcal infections in the USA, since staphylococci are still naïve to this agent and resistance is rarely observed among endemic *S. aureus*.

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