C1-1415

First Spontaneous Occurrence of High-Level Resistance to the Peptide Deformylase Inhibitor LBM415 in *Staphylococcus aureus* detected in a Clinical Setting

TR FRITSCHE, JA LEEDS, S ESTEROW, G DENYS, RN JONES

JMI Laboratories, North Liberty, IA; Novartis Institutes for Biomedical Research, Cambridge, MA; Clarian Health Partners, Inc., Indianapolis, IN

ICAAC 2005

JMI Laboratories
North Liberty, IA, USA
www.jmilabs.com
319.665.3370, fax 319.665.3371
ronald-jones@jmilabs.com

AMENDED ABSTRACT

Background: LBM415 was the first of the peptide deformylase (PDF) inhibitor (PDI) class to be advanced into clinical trials targeting community-acquired pathogens, including resistant Gram-positive cocci. To date, no strain of PDI-resistant (R) *S. aureus* (SA) has been reported in the > 9,000 strains tested in surveillance studies. This report describes the first PDI-R strain submitted as part of the 2004 LBM415 surveillance program.

Methods: SA was repeatedly isolated from a hospitalized patient with multiple fractures following a motor vehicle accident. Susceptibility (S) testing was performed using CLSI broth microdilution methods. *defB* and *fmt* genes were amplified from the index strain and from a PDI susceptible (S) strain using gene-specific primers, and sequenced.

Results: The isolate was found to be fully S to oxacillin and other agents except for penicillin and LBM415 (MIC, ≥ 1024 µg/ml). Sequence analysis revealed multiple nucleotide/amino acid substitutions in the formyltransferase gene *fmt*. In a laboratory setting, single-step *fmt* mutations in SA have been shown to result in loss-of-function. The *fmt* mutations identified here are consistent with the hypothesis that *fmt*-bypass in SA confers resistance to PDI. This strain also harbors a single nucleotide/amino acid substitution in the PDF *defB* gene. There are no previously reported data regarding selection of mutations in the SA *defB* gene upon exposure to PDI in the laboratory setting. Therefore, the contribution of this mutation to PDI-R is unknown.

Conclusions: Here we describe the first instance of a clinical SA strain R to LBM415. Mutations identified suggest that R occurs by a mechanism that has only been described in laboratory mutants. This finding is especially noteworthy in that no PDI compounds are in current clinical usage; furthermore, the patient did not participate in any PDI clinical trial.

INTRODUCTION

The emergence of antimicrobial resistance among the commonly occurring Gram-positive and –negative bacterial pathogens is a major health care crisis requiring a concerted response consisting of educational efforts to improve prescription discipline, improvements in infection control and public health infrastructure, expanded surveillance activities and, most urgently, development of new antimicrobials with novel mechanisms of action.

Inhibitors of bacterial peptide deformylase (PDF) are one class of novel agents currently in early stages of clinical development. Deformylase is essential for protein maturation in bacteria and the gene encoding PDF (*def*) has been found in all pathogens studied; no mammalian counterpart has been described, making it an attractive therapeutic target.

While a number of PDF inhibitors (PDI) have been described (actinonin was described in 1962), the Novartis Pharmaceuticals compound LBM415 has proceeded into Phase I clinical trials. This PDI has demonstrated potent activity (bacteriostatic) against Gram-positive pathogens (including methicillin-resistant staphylococci, penicillin-resistant pneumococci, and vancomycin-resistant enterococci) as well as against *Haemophilus influenzae* and *Moraxella catarrhalis*, and has been developed as an agent targeting community-acquired respiratory tract and skin and skin structure infections. When tested against large collections of wild-type staphylococci, no MIC above 4 µg/ml has been described.

Appearance of spontaneous mutations resulting in resistance to PDI has been detected among laboratory strains of *Staphylococcus aureus* (10^{-6} to 10^{-8} for both actinonin and LBM415) and *Streptococcus pneumoniae* (10^{-8} for actinonin). In *S. aureus,* resistance is due to inactivation of transformylase (Fmt) activity which renders deformylase non-essential. Interestingly, *S. aureus fmt* mutants grow more slowly than wild-type strains, and virulence is attenuated. Unlike *S. aureus*, an actinonin resistant *S. pneumoniae* strain was found to harbor a mutation in the deformylase gene (*defB*).

As part of global surveillance studies tracking the activity of LBM415 against contemporary pathogens, one *S. aureus* strain (out of > 9,000 tested) was found that exhibited high-level (≥ 1024 µg/ml) resistance. Given that no member of the PDI class has been introduced to date for clinical or veterinary use, the finding of a spontaneous resistant strain is of particular importance in helping define the genetic mechanisms of resistance that may be expected to appear once this class of agent is licensed.

MATERIALS AND METHODS

Study Strains: *S. aureus* strain 02-6390D was recovered repeatedly from chest and abdominal wounds, and bloodstream in a 34-year old patient hospitalized in 2004 who had experienced multiple fractures following a motor-vehicle accident. The patient was exposed to several classes of antibacterial agents during an extended hospital stay. A second *S. aureus* strain, NB01004, which is a fully sequenced Mu50 strain, was used as a sequence control.

Antimicrobial Susceptibility Testing: MIC values for LBM415 and 17 other antimicrobials were determined using validated, dry-form broth microdilution panels (TREK Diagnostics, Cleveland, OH) with cation-adjusted Mueller-Hinton broth medium according to the manufacturers recommendations and technical details of the CLSI (2003 and 2005). *S. aureus* ATCC 29213 was included as a quality control strain.

Amplification and Sequencing of *defB* and *fmt*: *defB* and *fmt* genes were amplified directly from a colony of 02-6390D grown on tryptic-soy agar containing 5% sheep blood, and from the frozen stock of NB01004, by PCR using the following parameters and conditions (per reaction [total 19 µl]): water, 12.4 µl; PCR buffer 4 µl of 5x stock; forward primer 1 µl of 100 µM stock; reverse primer 1 µl of 100 µM stock; dNTP 0.4 µl of 10 mM stock; and Phusion DNA polymerase, 0.2 µl of 2 U/µl stock (Finnzymes cat #F-530PS, lot #3).

The primers and amplification cycling conditions are as listed in Table 1. PCR products were examined on 1.2% agarose gels and then diluted 1:10 for sequencing. Sequencing was performed by Agencourt, Inc. (Bedford, MA).

RESULTS

- *S. aureus* strain 02-639D, a wild-type isolate recovered from a hospitalized patient, was fully resistant to the PDI LBM415 (≥ 1024 µg/ml); with the exception of penicillin resistance, the strain was fully susceptible to 16 other agents representing 10 antimicrobial classes (Table 2).
- This strain contained multiple sequence changes in genes relevant to the resistance phenotype (*defB* and *fmt*). These changes are outlined in Table 3.
- *fmt* sequence changes included L101I, a change in a highly conserved residue (*H. influenzae*, *P. aeruginosa*, *E. coli*, *S. aureus*, *E. faecalis*, *S. epidermidis*, but not *S. pneumoniae* or *B. subtilis*) at the N-terminal end of the Fmt active site.
- D195N is a *fmt* change in a functionally conserved residue (essentially it is either aspartic acid or glutamic acid in all relevant organisms) that is immediately next to an absolutely conserved aspartic acid.

Table 1. Details of the deformylase (*defB*) and formyltransferase (*fmt*) primers used in this study, and the PCR cycling conditions used for their amplifications.

Primers

Gene	Primer	Sequence
defB	BW15	5’GTTGTACAAGAAAAAGCTTAGGAC3’
	BW12	5’CTACAAAGTTAACCAACTACTGCC3’
fmt	BW23	5’GCATATTATAGATCAAATGAACGGT3’
	BW19	5’ATGAAGGTGCGTATAGTAACTTG3’

Amplification cycling parameters

Gene	Parameters
defB	1 x 10 min, 95°C; 30 x 30 sec, 95°C, 30 sec 53°C, 45 sec 72°C; 1 x 10 min, 72°C; 4°C hold
fmt	1 x 10 min, 95°C; 30 x 30 sec, 95°C, 30 sec 61°C, 65 sec 72°C; 1 x 10 min, 72°C; 4°C hold

- S212N is a third *fmt* change in a non-conserved residue that is found immediately after an absolutely conserved tryptophan.
- These *fmt* mutations are consistent with the hypothesis that Fmt-bypass in *S. aureus* confers resistance to LBM415 (the contribution of individual mutations has not been examined).
- A single *defB* mutation was detected in the C-terminal tail of the protein; this residue, while not conserved among the PDFs across species, is just downstream from the metal binding region of the enzyme.
- There are no data regarding the selection of mutations in *S. aureus defB*, therefore its contribution to PDI resistance remains unknown.

Table 2. Antibiogram for *Staphylococcus aureus* strain 02-639D, found to be resistant to the novel peptide deformylase inhibitor, LBM415.

	MIC (µg/ml)	
Antimicrobial agent	MIC (µg/ml)	Interpretive category:[a]
LBM415	≥1024	-
Oxacillin	0.5	S
Penicillin	0.5	R
Piperacillin/tazobactam	1/4	S
Ceftriaxone	2	S
Cefepime	2	S
Imipenem	≤0.12	S
Erythromycin	0.12	S
Clindamycin	0.12	S
Tetracycline	≤0.25	S
Ciprofloxacin	0.25	S
Levofloxacin	0.06	S
Trimethoprim/sulfamethoxazole	≤0.5	S
Rifampin	≤0.5	S
Quinupristin/dalfopristin	≤0.25	S
Linezolid	1	S
Vancomycin	1	S

a. Criteria are those of the CLSI [2005]; breakpoints for LBM415 have not been defined.

Table 3. Sequence changes identified in the *defB* and *fmt* genes of NB01087.

Gene	Nucleotide change*	Amino acid change
fmt	C301A	L101I
fmt	G583A	D195N
fmt	G635A	S212N
defB	A511G	N171D

*Numbering is according to Mu50 reference strain NC_002758

CONCLUSIONS

- We describe the first instance of a wild-type clinical *S. aureus* strain discovered to be resistant to a PDI, LBM415. The isolate was generally susceptible to all other tested antimicrobials.
- The *fmt* mutations identified are consistent with the hypothesis that Fmt-bypass in *S. aureus* confers resistance to PDI compounds (as described in laboratory mutants).
- The contribution of a mutation in the *defB* gene to resistance is unknown; there are no previously reported information regarding *defB* mutations in *S. aureus*.
- The mutations identified are especially noteworthy in that no PDI agents are currently in clinical usage; furthermore, the patient did not participate in any earlier PDI clinical trial.

SELECTED REFERENCES

Apfel CM, Locher H, Evers S, Takacs B, Hubschwerlen C, Pirson W, Page MGP, Keck W. (2001) Peptide deformylase as an antibacterial drug target: Target validation and resistance development. *Antimicrobial Agents and Chemotherapy* 45:1058-1064.

Clements JM, Beckett RP, Brown A, Catlin G, Lobell M, Palan S, Thomas W, Whittaker M, Wood S, Salama S, Baker PJ, Rodgers HF, Barynin V, Rice DW, Hunter MG. (2001) Antibiotic activity and characterization of BB-3497, a novel peptide deformylase inhibitor. *Antimicrobial Agents and Chemotherapy* 45:563-570.

Clinical and Laboratory Standards Institute. (2005) *Performance standards for antimicrobial susceptibility testing. Document M100-S15*. Wayne, PA:CLSI.

Credito K, Lin G, Ednie LM, Appelbaum PC. (2004) Antistaphylococcal activity of LBM415, a new peptide deformylase inhibitor, compared with those of other agents. *Antimicrobial Agents and Chemotherapy* 48:4033-4036.

Fritsche TR, Sader HS, Cleeland R, Jones RN. (2005) Comparative antimicrobial characterization of LBM415 (NVP PDF-713), a new peptide deformylase inhibitor of clinical importance. *Antimicrobial Agents and Chemotherapy* 49:1468-1476.

Giglione C, Pierre M, Meinnel T. (2000) Peptide deformylase as a target for new generation, broad spectrum antimicrobial agents. *Molecular Microbiology* 36:1197-1205.

Hackbarth CJ, Chen DZ, Lewis JG, Clark K, Mangold JB, Cramer JA, Margolis PS, wang W, Koehn J, Wu C, Lopez S, Withers G, Gu H, Dunn E, Kulathila R, Pan S-H, Porter WL, Jacobs J, Trias J, Patel DV, Weidmann B, White RJ, Yuan Z. (2002) *N*-Alkyl urea hydroxamic acids as a new class of peptide deformylase inhibitors with antibacterial activity. *Antimicrobial Agents and Chemotherapy* 46:2752-2764.

Margolis PS, Hackbarth C, Lopez S, Maniar M, Wang W, Yuan Z, White R, Trias J. (2001) Resistance of *Streptococcus pneumoniae* to deformylase inhibitors is due to mutations in *defB*. *Antimicrobial Agents and Chemotherapy* 45:2432-2435.

Margolis PS, Hackbarth CJ, Young DC, Wange W, Chen D, Yuan Z, White R, Trias J. (2000) Peptide deformylase in *Staphylococcus aureus*: Resistance to inhibition is mediated by mutations in the formyltransferase gene. *Antimicrobial Agents and Chemotherapy* 44:1825-1831.

National Committee for Clinical Laboratory Standards. (2003) *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Document M7-A6.* Wayne, PA:NCCLS.