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Molecular Characterization of Fluoroquinolone Resistance Mechanisms in Gram-Negative Isolates from the Delafloxacin Acute Bacterial Skin and Skin Structure Infections Clinical Trials

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Introduction

- Delafloxacin is a novel anionic fluoroquinolone antibiotic with broad-spectrum *in vitro* activity, including activity against Gram-positive organisms, Gram-negative organisms, anaerobes, and atypical respiratory tract pathogens (ie, *Legionella*, *Chlamydia*, and *Mycoplasma*)
- This fluoroquinolone was recently (June 2017) approved by the Food and Drug Administration (FDA) to treat acute bacterial skin and skin structure infections (ABSSSI)
  - FDA approval was based on delafloxacin noninferiority to vancomycin plus aztreonam to treat ABSSSI in patients enrolled in 2 (RX-3341-302 and RX-3341-303) global phase 3 studies
- During these phase 3 studies, 13.2% of patients enrolled in the delafloxacin arm (pooled data) had polymicrobial infections consisting of Gram-positive and Gram-negative organisms (Table 1)
- This study characterized the fluoroquinolone resistance mechanisms among Gram-negative clinical isolates recovered from the microbiologically evaluable (ME) population and associated with ABSSSI and correlated with microbiologic response

Materials and Methods

Subjects and clinical isolates

- A total of 1,510 subjects were enrolled in the delafloxacin phase 3 trials for ABSSSI
  - Patients were hospitalized in medical sites located in 23 countries, including the United States (62.3%), and countries in Europe (30.2%), South America (6.0%), and Asia (1.5%)
  - The enrollment period spanned from April 2013 to January 2016
- The microbiological intent-to-treat (MITT) population consisted of 1,042 subjects (n = 518 subjects in the delafloxacin arm; n = 524 subjects in the vancomycin-plus-aztreonam arm)
- The microbiologically evaluable at follow-up (MEFU) consisted of 806 subjects (n = 410 subjects in the delafloxacin arm; n = 396 subjects in the vancomycin-plus-aztreonam arm)

Table 1 Monomicrobial and polymicrobial infections in the MEFU populations at baseline by ABSSSI study

MEFUI	Delafloxacin N (%; N/N1) by study		
	RX-3341-302	RX-3341-303	Pool 1
All Infection Types, N1	**179**	**231**	**410**
Monomicrobial			
Gram-positive	126 (70.4)	144 (62.3)	270 (65.9)
Gram-negative	6 (3.4)	10 (4.3)	16 (3.9)
Polymicrobial			
Gram-positive	24 (13.4)	41 (17.7)	65 (15.9)
Gram-negative		5 (2.2)	5 (1.2)
Gram-positive and -negative	23 (12.8)	31 (13.4)	54 (13.2)
Aerobe and anaerobe	9 (5.0)	10 (4.3)	19 (4.6)

ABSSSI, acute bacterial skin and skin structure infection; MEFU, microbiologically evaluable at follow-up for the investigator-assessed response

- For this study, a total of 18 *Enterobacteriaceae* and 11 *Pseudomonas aeruginosa* baseline isolates showing delafloxacin MIC values of ≥0.12 µg/mL, recovered from the delafloxacin study arm, were included

Microbiology and clinical outcomes

- The microbiologic responses of patients in the ME and MITT analysis data sets were based on the results of baseline and postbaseline cultures (follow-up [FU] and late follow-up [LFU]) and susceptibility testing, together with the clinical response assigned by the investigator

Antimicrobial susceptibility testing

- Susceptibility testing of clinical isolates was centrally performed using the broth microdilution method following the Clinical Laboratory Standards Institute (CLSI) guidelines (M07-A10)
- Quality assurance was performed by concurrently testing CLSI-recommended quality control reference strains
  - All QC results were within published acceptable ranges
- Delafloxacin breakpoints published by the FDA were applied for *Enterobacteriaceae* and *P. aeruginosa*

Screening of fluoroquinolone resistance mechanisms

- Total genomic DNA was extracted using the Thermo Scientific™ KingFisher™ Flex Magnetic Particle Processor (Cleveland, Ohio, USA)
- Total genomic DNA samples were used as input material for library construction
  - DNA libraries were prepared using the NexteraXT™ library construction protocol (Illumina, San Diego, California, USA), following the manufacturer's instructions, and sequenced on a MiSeq Sequencer (JMI Laboratories, North Liberty, Iowa, USA)
- Assembled genomes were subjected to a proprietary software (JMI Laboratories) for screening acquired resistance genes (Table 2)
  - Genomes were blasted against plasmid-mediated genes associated with fluoroquinolone resistance present in a curated database

Table 2 Plasmid-mediated genes encoding for the following proteins were screened in this study

Fluoroquinolone resistance mechanism							
Efflux pump	Target protection						Aminoglycoside
OqxA11	QnrA1	QnrB17	QnrB31	QnrB47	QnrB61	QnrC	AAC(6')Ib-cr
OqxA-2	QnrA2	QnrB18	QnrB32	QnrB48	QnrB62	QnrD	
OqxA-2-like	QnrA3	QnrB19	QnrB33	QnrB49	QnrB64	QnrS1	
OqxB	QnrA4	QnrB2	QnrB34	QnrB5	QnrB65	QnrS2	
OqxB19	QnrA5	QnrB20	QnrB35	QnrB50	QnrB66	QnrS4	
OqxB20	QnrA6	QnrB21	QnrB36	QnrB51	QnrB67	QnrS5	
OqxB32	QnrA7	QnrB22	QnrB37	QnrB52	QnrB68	QnrS6	
OqxB-like	QnrAS	QnrB23	QnrB38	QnrB53	QnrB69	QnrVC1	
QepA	QnrB1	QnrB24	QnrB4	QnrB54	QnrB6-	QnrVC3	
QepA2	QnrB10	QnrB25	QnrB41	QnrB56	QnrB7	QnrVC4	
	QnrB11	QnrB27	QnrB42	QnrB57	QnrB70	QnrVC5	
	QnrB12	QnrB28	QnrB43	QnrB58	QnrB71	QnrVC7	
	QnrB13	QnrB29	QnrB44	QnrB59	QnrB72		
	QnrB14	QnrB3	QnrB45	QnrB6	QnrB8		
	QnrB15	QnrB30	QnrB46	QnrB60	QnrB9		

- GyrA and GyrB (encode for DNA gyrase) and ParC and ParE (encode for topoisomerase IV) sequences were extracted from assembled genomes and screened for mutations in the quinolone-resistance determinant regions (QRDRs)
- The transcription levels of efflux-pump (MexAB, MexCD, MexEF, and MexXY) genes were determined using quantitative real-time PCR assays (qRT-PCR)
  - During the quantification process for the target mRNA gene, a normalized expression analysis method was applied and relative comparison to a susceptible control strain was performed
  - A strain was considered to overexpress efflux-pump genes when at least a 5-fold greater difference of transcripts was detected as compared with a wild-type reference control strain

Results

- All subjects infected with Gram-negative isolates tested with delafloxacin MIC results of ≥0.12 µg/mL had polymicrobial infections, except for 4 patients (Table 3)
- *Enterobacteriaceae* isolates exhibiting delafloxacin MIC values of 0.12–0.5 µg/mL did not show any of the investigated fluoroquinolone resistance mechanisms (Table 3)
- *E. cloacae* isolates (delafloxacin MIC, 2–4 µg/mL) harbored a GyrA alteration (S83T) or *qnrB6*, while *Escherichia coli* (delafloxacin MIC, 1–2 µg/mL) had multiple mutations in GyrA, ParE, and ParC or *qnrS1* (Table 3)
- All *Klebsiella pneumoniae* and *Proteus mirabilis* (delafloxacin MIC, 1–4 µg/mL) isolates had mutations in GyrA at serine 83 and ParC at serine 80 or 84 (Table 3)
  - One *P. mirabilis* isolate displaying a delafloxacin MIC of >8 µg/mL also carried *qnrA1* in addition to QRDR mutations
- *P. aeruginosa* isolates tested with a delafloxacin MIC of 0.12–0.5 µg/mL did not show any of the investigated fluoroquinolone resistance mechanisms
- *P. aeruginosa* isolates displaying a delafloxacin MIC of 1 µg/mL had a GyrA alteration at position 87, while those with an MIC of 4 µg/mL had overexpression of the MexA efflux pump (Tables 3 and 4)
- All subjects where Gram-negative isolates tested with delafloxacin MIC results of ≥0.12 µg/mL showed microbiologic eradication at FU except for 1 subject with a monomicrobial infection with persistent *E. cloacae* (isolate #382)

Table 3 Delafloxacin, ciprofloxacin, and levofloxacin MIC results, fluoroquinolone resistance mechanism and microbiologic responses[a]

Isolate no.	Organism evaluated	MIC (µg/mL)[b]			QRDR[c]				Plasmid-mediated	Organism present if polymicrobial infection (delafloxacin MIC, µg/mL)[a]
		DEL	CIP	LEV	GyrA	GyrB	ParC	ParE		
382	*E. cloacae*	2	0.12	0.25	S83T	WT	WT	WT		
1109		4	4	0.5	WT	WT	WT	WT	*qnrB6*	
823	*E. coli*	1	0.5	0.5	WT	WT	WT	WT	*qnrS1*	*S. pyogenes* (0.03)
557		2	>8	>8	S83L, D87N	WT	S80I	S458A		*S. agalactiae* (0.015)
1118	*K. oxytoca*	0.25	≤0.06	0.12	WT	WT	WT	WT		*S. anginosus* (0.03)
611		0.25	0.25	1	WT	WT	WT	WT		*S. aureus* (0.002) – *P. acne* (NA)
757	*K. pneumoniae*	0.12	≤0.06	0.12	WT	WT	WT	WT		*P. mirabilis* (0.06)
734		0.12	≤0.06	0.12	WT	WT	WT	WT		*S. aureus* (0.008)
104		0.12	≤0.06	0.12	WT	WT	WT	WT		*Peptoniphilus* spp. (NA)
260		0.12	0.12	≤0.06	WT	WT	WT	WT		
426		0.25	≤0.06	≤0.06	WT	WT	WT	WT		*S. constellatus* (NA)
31		0.25	≤0.06	0.12	WT	WT	WT	WT		*S. aureus* (0.25)
947		0.25	0.12	0.5	WT	WT	WT	WT		*S. aureus* (0.25)
496		4	>8	8	S83I	WT	S80I	WT		*S. agalactiae* (0.03)
840	*P. mirabilis*	0.12	0.12	0.25	WT	WT	WT	WT		*E. faecalis* (0.12)
326		1	1	1	S83I	WT	S84R	WT		*S. dysgalactiae* (0.015)
771		>8	>8	>8	S83I	WT	S84I	WT	*qnrA1*	
719		2	4	2	S83I	WT	S84R	WT		*S. aureus* (0.002), *E. faecalis* (0.12)
724	*P. aeruginosa*	0.12	≤0.06	0.25	WT	WT	WT	WT		*S. aureus* (0.002), *E. faecalis* (0.12)
555		0.12	≤0.06	0.25	WT	WT	WT	WT		*S. aureus* (0.008), *S. dysgalactiae* (0.008)
4		0.25	0.12	0.5	WT	WT	WT	WT		*Serratia marcescens* (NA)
390		0.25	0.12	0.5	WT	WT	WT	WT		*S. aureus* (0.008)
915		0.25	0.12	0.25	WT	WT	WT	WT		*S. haemolyticus* (0.008)
58		0.25	0.12	0.5	WT	WT	WT	WT		*S. aureus* (0.015)
125		0.5	0.12	0.5	WT	WT	WT	WT		*S. aureus* (0.12)
280		0.5	0.5	0.5	WT	WT	WT	WT		*S. dysgalactiae* (0.015)
313		1	0.5	2	D87N	WT	WT	WT		*S. dysgalactiae* (0.015)
140		4	1	8	WT	WT	WT	WT		*S. aureus* (0.002), *E. cloacae* (0.06)
123		4	1	8	WT	WT	WT	WT		*S. aureus* (0.5)

[a] All isolates were eradicated at follow-up visit or late follow-up visit, except for isolate #1109 (persistence) causing a monomicrobial infection
[b] DEL, delafloxacin; CIP, ciprofloxacin; LEV, levofloxacin
[c] Quinolone-resistance determinant regions (QRDRs) within GyrA and GyrB (encode for DNA gyrase) and ParC and ParE (encode for topoisomerase IV) genes; WT, wild type
[d] Additional organisms recovered from the same patient/clinical specimen; NA, MIC not available

Table 4 Transcription levels of constitutive efflux-pump systems in *P. aeruginosa*

Subject	Isolate no.	MIC (µg/mL)[a]			QRDR[b]				Relative expression of efflux pumps[c]			
		DEL	CIP	LEV	GyrA	GyrB	ParC	ParE	MexA	MexC	MexE	MexX
840-323-3147	125	0.5	0.12	0.5	WT	WT	WT	WT	2.62	0.74	0.07	1.85
410-338-3277	280	0.5	0.5	0.5	WT	WT	WT	WT	1.23	1.78	0.02	0.74
233-441-3350	313	1	0.5	2	D87N	WT	WT	WT	0.54	0.49	0.04	0.29
840-321-3022	140	4	1	8	WT	WT	WT	WT	**5.67**	0.51	<0.01	0.64
840-321-3024	123	4	1	8	WT	WT	WT	WT	**6.73**	1.53	0.01	1.07

[a] DEL, delafloxacin; CIP, ciprofloxacin; LEV, levofloxacin
[b] Quinolone-resistance determinant regions (QRDRs) within GyrA and GyrB (encode for DNA gyrase) and GrlA (ParC) and GrlB (ParE) (encode for topoisomerase IV) genes; WT, wild type
[c] Expression levels (transcription rates) of efflux-pump genes relative to an internal control gene *rpsL* and compared to a wild-type control strain (PA01); results in bold may be considered moderate expression

Conclusions

- Fluoroquinolone resistance mechanisms present in these selected Gram-negative isolates were associated with delafloxacin MIC values of ≥1 µg/mL
- All patients included in this study had favorable microbiologic response (eradication) at FU, except for 1 subject with a persistent *E. cloacae* (delafloxacin MIC, 2 µg/mL) harboring a GyrA mutation
  - Eradication or presumed eradication was observed in subjects with isolates with delafloxacin MIC values as high as >8 µg/mL
- The presence of polymicrobial infection and/or isolates with fluoroquinolone resistance determinants do not appear to affect delafloxacin microbiologic responses when treating ABSSSI

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