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Activity of Pexiganan when Tested against Contemporary Gram-Positive, Gram-Negative Bacteria and Yeast Collected from North America, Europe and Japan

RK FLAMM, HS SADER, PR RHOMBERG

JMI Laboratories, North Liberty, IA, USA

Robert Flamm, PhD
JMI Laboratories
335 Beaver Kreek Centre
North Liberty, Iowa 52317
Phone: (319) 665-3370
robert-flamm@jmilabs.com

Amended Abstract

Background: Pexiganan is a synthetic analogue of peptide Magainin II in Phase 3 clinical trials as a topical cream for the treatment of mild infections of diabetic foot ulcers. The aim of this study was to evaluate the current *in vitro* activity of pexiganan against bacteria and yeast from North America, Europe and Japan.

Methods: A total of 2,382 Gram-positive (GP), 1,721 Gram-negative (GN) and 205 *Candida albicans* isolates (94.2% from 2014-15) were tested for susceptibility (S) to pexiganan and comparators by CLSI and/or EUCAST reference broth microdilution methods. Isolates were from multiple sources of infection including skin and soft tissue, bloodstream, respiratory, and others.

Results: Pexiganan was active against *Staphylococcus aureus* (SA; $MIC_{50/90}$, 8/16 µg/mL; highest MIC, 32 µg/mL). The $MIC_{50/90}$ for methicillin-resistant (MRSA; 40.0% of SA) and methicillin-susceptible (MSSA) isolates were identical at 8/16 µg/mL. All SA were S to vancomycin (VAN; $MIC_{50/90}$, 0.5/1 µg/mL) and linezolid ($MIC_{50/90}$, 1/1 µg/mL), while 99.9% were S to daptomycin ($MIC_{50/90}$, 0.25/0.5 µg/mL). Pexiganan showed 64- to 128-fold greater potency against *E. faecium* ($MIC_{50/90}$, 4/4 µg/mL) than *E. faecalis* ($MIC_{50/90}$, 256/512 µg/mL). Against β-hemolytic streptococci (BHS), pexiganan showed a MIC range of 2-16 µg/mL ($MIC_{50/90}$, 4/16 µg/mL). Among Enterobacteriaceae, pexiganan showed activity against *Citrobacter* spp., *Escherichia coli*, *Klebsiella* spp. and *Enterobacter* spp. ($MIC_{50/90}$ range, 2-16/4-128 µg/mL, respectively). MICs were higher for *P. mirabilis*, indole-positive Proteeae and *S. marcescens* ($MIC_{50/90}$, >1024/>1024 µg/mL). Pexiganan was active against non-fermentative GN bacilli as well as yeast. $MIC_{50/90}$ values were: *Acinetobacter* spp. ($MIC_{50/90}$, 4/4 µg/mL), *Pseudomonas aeruginosa* ($MIC_{50/90}$, 8/16 µg/mL), *Stenotrophomonas maltophilia* ($MIC_{50/90}$, 4/16 µg/mL) and *Candida albicans* ($MIC_{50/90}$,32-128/128 µg/mL).

Conclusions: Pexiganan was active against a broad range of microorganisms including MRSA, VAN-R enterococci and BHS. Pexiganan is an important new therapeutic agent in the current environment of emerging multi-drug resistant pathogens.

Introduction

- Pexiganan is a synthetic analogue of peptide Magainin II in Phase 3 clinical trials as a topical cream for the treatment of mild infections of diabetic foot ulcers.
- The aim of this study was to evaluate the current *in vitro* activity of pexiganan against bacteria and yeast from North America, Europe and Japan collected within the last 3 years.
- 4,308 isolates were selected for the study including Gram-positive cocci (n=2,382), Gram-negative bacilli (n=1,721) and yeast (n=205).
- All isolates were from the SENTRY surveillance program; 94.2% were collected in 2014-2015.

Methods

- MIC values for bacteria were determined using Clinical and Laboratory Standards Institute (CLSI) broth microdilution methodology. MIC values for yeast were determined using CLSI and/or EUCAST broth microdilution methods. Quality control of susceptibility testing was performed according to CLSI.
- Pexiganan and comparator agents were tested in 96-well, frozen-form panels produced by JMI Laboratories (North Liberty, Iowa, USA) with three media types: 1) cation-adjusted Mueller-Hinton broth (CA-MHB); 2) CA-MHB supplemented with 3.75% lysed horse blood for streptococci, and; 3) RPMI broth for yeast. MIC values for yeast were recorded at both 24 and 48 hours. CLSI (5,6) and EUCAST (7) breakpoints were used for comparators.
- Resistant subsets tested in this study were identified as follows:
  1. “ESBL-phenotype” was defined as an MIC value of ≥2 µg/mL for ceftriaxone and/or ceftazidime and/or aztreonam (5) for the following organisms: *Escherichia coli, Klebsiella pneumoniae, K. oxytoca* and *Proteus mirabilis*.
  2. “Carbapenem-resistant Enterobacteriaceae (CRE)” was defined as an isolate displaying an MIC value of ≥4 µg/mL (5) for imipenem and/or meropenem and/or doripenem (*Proteus mirabilis* and indole-positive Proteeae were not included due to their intrinsically elevated MIC values).
  3. *Acinetobacter baumannii* was classified as multi-drug resistant (MDR) if it was non-susceptible to three or more classes of antibiotics.

Results

- The isolates in this study were all clinical isolates. The most common infection source was skin/skin structure infection (28%), bloodstream infections were second (26%) followed by pneumonia in hospitalized patients (15%).
- Gram-positive cocci (staphylococci, streptococci and enterococci) were the main pathogens isolated from skin/skin structure and bloodstream infections, while Gram-negatives were more common pneumonia isolates.
- **Table 1** shows the activity of pexiganan against the main bacterial organism groups and resistant sub-groups tested in this study.
- Pexiganan was active against *Staphylococcus aureus* ($MIC_{50/90}$, 8/16 µg/mL; highest MIC, 32 µg/mL; **Table 1**). The $MIC_{50/90}$ for methicillin-resistant (MRSA; 40.0% of *S. aureus*) and methicillin-susceptible (MSSA) isolates were identical at 8/16 µg/mL.
- All *S. aureus* (**Table 2**) were susceptible to vancomycin ($MIC_{50/90}$, 0.5/1 µg/mL) and linezolid ($MIC_{50/90}$, 1/1 µg/mL), while 99.9% were susceptible to daptomycin ($MIC_{50/90}$, 0.25/0.5 µg/mL).
- For MRSA (**Table 2**), susceptibility for the comparators ranged from 18.2% levofloxacin susceptible to 100% vancomycin and linezolid susceptible. As stated above, pexiganan had similar MIC values regardless of the presence of various resistance determinants.
- Pexiganan showed 64- to 128-fold greater potency against *E. faecium* ($MIC_{50/90}$, 4/4 µg/mL) than *E. faecalis* ($MIC_{50/90}$, 256/512 µg/mL, **Table 1**). Vancomycin resistance (vanA/B) had no effect on pexiganan MIC.
- Against β-hemolytic streptococci, pexiganan showed a MIC range of 2-16 µg/mL ($MIC_{50/90}$, 4/16 µg/mL; **Table 1**).
- Among Enterobacteriaceae, pexiganan showed activity against *Citrobacter* spp., *Escherichia coli*, *Klebsiella* spp. and *Enterobacter* spp. ($MIC_{50/90}$, range from 2-16/4-128 µg/mL, respectively; **Table 1**).
- Activity of pexiganan was unaffected by the presence of extended-spectrum beta-lactamase or carbapenemase (**Table 1**). The single CRE isolate with elevated pexiganan MIC was a *S. marcescens,* which show inherently higher MICs for pexiganan (**Table 1**).
- All *E. coli* isolates tested were susceptible to colistin ($MIC_{50/90}$, ≤0.5/≤0.5 µg/mL) and meropenem ($MIC_{50/90}$, 0.015/0.03 µg/mL) while 67.0% were susceptible to trimethoprim-sulfamethoxazole and 73.9% were susceptible to levofloxacin (**Table 3**). The different resistance mechanisms present had no impact on pexiganan activity ($MIC_{50/90}$, 8/16 µg/mL).
- **Table 3** shows MIC values for pexiganan and comparators when tested against 203 *K. pneumoniae* isolates. There were five colistin-resistant isolates (97.5% colistin-susceptible). The isolates had colistin MICs 8->8 µg/mL with a pexiganan MIC range of 8-64 µg/mL. Pexiganan activity was consistent against isolates that were resistant to other drug classes including fluoroquinolones and carbapenems.
- **Figure 1** shows pexiganan activity against 31 colistin-resistant Gram-negative isolates included in this study. The pexiganan MICs ranged from 4-1024 µg/mL demonstrating a lack of cross resistance with colistin. **Figure 1** excludes *Morganella, Proteus* and *Serratia* species, which have inherently higher MICs for pexiganan ($MIC_{50/90}$, >1024/>1024 µg/mL, **Table 1**) and to colistin.
- Pexiganan was active against non-fermentative Gram-negative bacilli (**Table 1**). $MIC_{50/90}$ values were: *Acinetobacter* spp. ($MIC_{50/90}$, 4/4 µg/mL), *Pseudomonas aeruginosa* ($MIC_{50/90}$, 8/16 µg/mL) and *Stenotrophomonas maltophilia* ($MIC_{50/90}$, 4/16 µg/mL).
- Pexiganan also showed good activity against 205 isolates of *Candida albicans* (**Table 4**). The pexiganan MICs ranged from 4 to 128 µg/mL when tested with the CLSI method, with a $MIC_{50/90}$ of 32/32 µg/mL when read at 24 hrs and 32/128 µg/mL when read at 48 hrs. When tested with the EUCAST method, the MIC range was 16-256 µg/mL ($MIC_{50/90}$, 128/128 µg/mL).

Table 1. Antimicrobial activity of pexiganan tested against the main organisms and organism groups of isolates included in this study.

Organisms / Organism Groups	No. of isolates at MIC (µg/mL; cumulative %)													MIC$_{50}$	MIC$_{90}$
	0.5	1	2	4	8	16	32	64	128	256	512	1024	>1024		
CRE (21)			0 (0.0%)	3 (14.3%)	9 (57.1%)	6 (85.7%)	0 (85.7%)	2 (95.2%)	0 (95.2%)	0 (95.2%)	0 (95.2%)	0 (95.2%)	1 (100.0%)	8	64
Escherichia coli (203)			9 (4.4%)	55 (31.5%)	110 (85.7%)	26 (98.5%)	3 (100.0%)							8	16
ESBL-phenotype (30)			0 (0.0%)	1 (3.3%)	10 (36.7%)	18 (96.7%)	1 (100.0%)							8	8
Klebsiella pneumoniae (203)			0 (0.0%)	5 (2.5%)	54 (29.1%)	95 (75.9%)	32 (91.6%)	14 (98.5%)	3 (100.0%)					8	16
ESBL-phenotype (46)				0 (0.0%)	11 (23.9%)	23 (73.9%)	9 (93.5%)	1 (95.7%)	2 (100.0%)					8	16
Klebsiella oxytoca (103)			0 (0.0%)	2 (1.9%)	73 (72.8%)	24 (96.1%)	4 (100.0%)							4	8
ESBL-phenotype (9)				0 (0.0%)	7 (77.8%)	2 (100.0%)								4	--
Enterobacter cloacae (102)			0 (0.0%)	2 (2.0%)	31 (32.4%)	34 (65.7%)	8 (73.5%)	8 (81.4%)	6 (87.3%)	6 (93.1%)	2 (95.1%)	4 (99.0%)	1 (100.0%)	8	128
Enterobacter aerogenes (103)				0 (0.0%)	3 (2.9%)	47 (48.5%)	38 (85.4%)	12 (97.1%)	2 (99.0%)	1 (100.0%)				16	32
Serratia marcescens (103)					0 (0.0%)	1 (1.0%)	2 (2.9%)	1 (3.9%)	0 (3.9%)	0 (3.9%)	0 (3.9%)	1 (4.9%)	1 (5.8%)	97 (100.0%)	>1024	>1024
Proteus mirabilis (101)			0 (0.0%)	1 (1.0%)	1 (2.0%)	2 (5.9%)	3 (8.9%)	3 (11.9%)	3 (15.8%)	2 (17.8%)	1 (18.8%)	1 (19.8%)	81 (100.0%)	>1024	>1024
Proteus vulgaris (101)			0 (0.0%)	1 (1.0%)	0 (1.0%)	3 (4.0%)	0 (4.0%)	0 (4.0%)	0 (4.0%)	0 (4.0%)	1 (5.0%)	1 (5.9%)	95 (100.0%)	>1024	>1024
Citrobacter koseri (100)		0 (0.0%)	51 (51.0%)	46 (97.0%)	3 (100.0%)									2	4
Citrobacter freundii species complex (102)		0 (0.0%)	13 (12.7%)	85 (96.1%)	3 (99.0%)	0 (99.0%)	1 (100.0%)							4	4
Morganella morganii (100)			0 (0.0%)	1 (1.0%)	3 (4.0%)	0 (4.0%)	1 (5.0%)	0 (5.0%)	0 (5.0%)	0 (5.0%)	1 (6.0%)	1 (7.0%)	0 (7.0%)	93 (100.0%)	>1024	>1024
Pseudomonas aeruginosa (200)			0 (0.0%)	5 (2.5%)	27 (16.0%)	142 (87.0%)	25 (99.5%)	1 (100.0%)						8	16
meropenem-non-susceptible (MIC, ≥4 µg/mL) (45)			0 (0.0%)	3 (6.7%)	6 (20.0%)	29 (84.4%)	6 (97.8%)	1 (100.0%)						8	16
ceftazidime-non-susceptible (MIC, ≥16 µg/mL) (49)			0 (0.0%)	3 (6.1%)	7 (20.4%)	33 (87.8%)	6 (100.0%)							8	16
Acinetobacter baumannii (101)		0 (0.0%)	1 (1.0%)	30 (30.7%)	65 (95.0%)	5 (100.0%)								4	4
MDR (52)			0 (0.0%)	19 (36.5%)	31 (96.2%)	2 (100.0%)								4	4
Stenotrophomonas maltophilia (99)			0 (0.0%)	39 (39.4%)	27 (66.7%)	18 (84.8%)	10 (94.9%)	0 (94.9%)	3 (98.0%)	2 (100.0%)				4	16
Staphylococcus aureus (893)			0 (0.0%)	6 (0.7%)	194 (22.4%)	503 (78.7%)	159 (96.5%)	31 (100.0%)						8	16
oxacillin-resistant (357)			0 (0.0%)	1 (0.3%)	60 (17.1%)	205 (74.5%)	73 (95.0%)	18 (100.0%)						8	16
Coagulase-negative staphylococci (495)		0 (0.0%)	83 (16.8%)	385 (94.5%)	9 (96.4%)	18 (100.0%)								2	2
oxacillin-resistant (308)		0 (0.0%)	42 (13.6%)	246 (93.5%)	5 (95.1%)	15 (100.0%)								2	2
β-haemolytic streptococci (457)			0 (0.0%)	8 (1.8%)	247 (55.8%)	153 (89.3%)	49 (100.0%)							4	16
Streptococcus pneumoniae (99)				1 (0.0%)	7 (1.0%)	26 (8.1%)	36 (15.2%)	29 (51.5%)	15 (80.8%)	3 (96.0%)	1 (99.0%)	1 (100.0%)		32	128
Viridans group streptococci (142)				0 (0.0%)	12 (8.5%)	26 (26.8%)	18 (39.4%)	20 (53.5%)	30 (74.6%)	22 (90.1%)	7 (95.1%)	5 (98.6%)	2 (100.0%)	32	128
Enterococcus spp. (296)		0 (0.0%)	2 (0.7%)	59 (20.6%)	71 (44.6%)	6 (46.6%)	1 (47.0%)	3 (48.0%)	19 (54.4%)	39 (67.6%)	45 (82.8%)	51 (100.0%)		64	512
Enterococcus faecalis (158)					0 (0.0%)	1 (0.6%)	3 (2.5%)	19 (14.6%)	39 (39.2%)	45 (67.7%)	51 (100.0%)			256	512
Enterococcus faecium (137)		0 (0.0%)	2 (1.5%)	59 (44.5%)	70 (95.6%)	6 (100.0%)								4	4
vanB (9)			0 (0.0%)	5 (55.6%)	4 (100.0%)									2	--
vanA (85)			0 (0.0%)	42 (49.4%)	1 (98.8%)	1 (100.0%)								4	4

Table 2. Activity of pexiganan and comparator antimicrobial agents when tested against *S. aureus* (n=893) and oxacillin resistant *S. aureus* (MRSA, n=357).

Antimicrobial Agent	MIC$_{50}$	MIC$_{90}$	Range	CLSI[a] %S	CLSI[a] %I	CLSI[a] %R	EUCAST[a] %S	EUCAST[a] %I	EUCAST[a] %R
Pexiganan- All	8	16	2 — 32	-	-	-	-	-	-
MRSA	8	16	2 — 32	-	-	-	-	-	-
Clindamycin- All	≤0.25	>2	≤0.25 — >2	83.3	0.3	16.3	83.0	0.3	16.7
MRSA	≤0.25	>2	≤0.25 — >2	64.1	0.3	35.6	63.9	0.3	35.9
Daptomycin- All	0.25	0.5	≤0.12 — 2	99.9	-	-	99.9	-	0.1
MRSA	0.25	0.5	≤0.12 — 2	99.7	-	-	99.7	-	0.3
Erythromycin- All	0.5	>8	≤0.12 — >8	52.6	5.2	42.2	53.3	1.3	45.4
MRSA	>8	>8	≤0.12 — >8	20.4	5.6	73.9	21.6	1.7	76.8
Levofloxacin- All	0.25	>16	0.06 — >16	62.2	0.4	37.4	62.2	0.4	37.4
MRSA	16	>16	0.12 — >16	18.2	0.6	81.2	18.2	0.6	81.2
Linezolid- All	1	1	≤0.12 — 2	100.0	-	0.0	100.0	-	0.0
MRSA	1	1	0.25 — 2	100.0	-	0.0	100.0	-	0.0
Meropenem- All	0.12	>16	0.03 — >16	-	-	-	-	-	-
MRSA	8	>16	0.12 — >16	-	-	-	-	-	-
Tetracycline- All	≤0.5	4	≤0.5 — >8	90.1	0.7	9.2	89.1	0.8	10.1
MRSA	≤0.5	>8	≤0.5 — >8	81.5	0.6	17.9	80.4	1.1	18.5
TMP-SMX[c]- All	≤0.5	≤0.5	≤0.5 — >4	98.9	-	1.1	98.9	0.2	0.9
MRSA	≤0.5	≤0.5	≤0.5 — >4	98.0	-	2.0	98.0	0.3	1.7
Vancomycin- All	0.5	1	≤0.12 — 2	100.0	0.0	0.0	100.0	-	0.0
MRSA	0.5	1	≤0.12 — 2	100.0	0.0	0.0	100.0	-	0.0

a. Criteria as published by CLSI [2016] and EUCAST [2016]
b. Oxacillin non-susceptible reported as resistant
c. Trimethoprim-sulfamethoxazole

Table 3. Activity of pexiganan and comparator antimicrobials against *E. coli* and *K. pneumoniae.*

Antimicrobial Agent	MIC$_{50}$	MIC$_{90}$	Range	CLSI[a] %S	CLSI[a] %I	CLSI[a] %R	EUCAST[a] %S	EUCAST[a] %I	EUCAST[a] %R
E. coli (n=203)									
Pexiganan	8	16	2 — 32	-	-	-	-	-	-
Amikacin	2	4	0.5 — 32	99.5	0.5	0.0	99.0	0.5	0.5
Amox-clav[b]	8	>8	≤1 — >8	78.8	-	-[c]	78.8	-	21.2
Ceftazidime	0.12	4	0.03 — >32	91.1	1.5	7.4	86.2	4.9	8.9
Ceftriaxone	≤0.06	>8	≤0.06 — >8	87.7	0.5	11.8	87.7	0.5	11.8
Colistin	≤0.5	≤0.5	≤0.5 — 2	-	-	-	100.0	-	0.0
Gentamicin	≤1	4	≤1 — >8	90.6	0.0	9.4	89.7	1.0	9.4
Levofloxacin	0.03	16	0.015 — >16	73.9	3.0	23.2	73.9	0.0	26.1
Meropenem	0.015	0.03	0.015 — 0.12	100.0	0.0	0.0	100.0	0.0	0.0
Tetracycline	2	>8	≤0.5 — >8	65.0	1.0	34.0	-	-	-
TMP-SMX[d]	≤0.5	>4	≤0.5 — >4	67.0	-	33.0	67.0	0.0	33.0
K. pneumoniae (n=203)									
Pexiganan	8	16	2 — 64	-	-	-	-	-	-
Amikacin	1	4	≤0.25 — 32	95.1	4.9	0.0	94.6	0.5	4.9
Amox-clav	2	>8	≤1 — >8	77.8	-	-[c]	77.8	-	22.2
Ceftazidime	0.12	32	0.03 — >32	81.8	2.5	15.8	78.8	3.0	18.2
Ceftriaxone	≤0.06	>8	≤0.06 — >8	78.8	0.0	21.2	78.8	0.0	21.2
Colistin	≤0.5	1	≤0.5 — >8	-	-	-	97.5	-	2.5
Gentamicin	≤1	>8	≤1 — >8	87.2	0.5	12.3	87.2	0.0	12.8
Levofloxacin	0.06	>16	0.03 — >16	83.3	1.0	15.8	82.8	0.5	16.7
Meropenem	0.03	0.06	0.015 — >16	91.1	1.5	7.4	92.6	2.5	4.9
Tetracycline	1	>8	≤0.5 — >8	76.8	4.4	18.7	-	-	-
TMP-SMX	≤0.5	>4	≤0.5 — >4	74.4	-	25.6	74.4	1.0	24.6

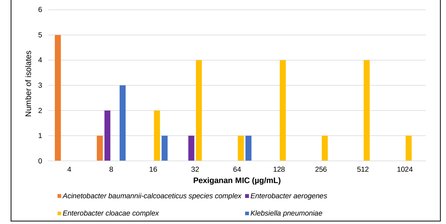
a. Criteria as published by CLSI [2016] and EUCAST [2016]
b. Amoxicillin-clavulanic acid
c. Dilution range did not extend high enough to determine between I and R so only Susceptible Percentage is displayed
d. Trimethoprim-sulfamethoxazole

Table 4. Antimicrobial activity of pexiganan against *Candida albicans* isolates.

Organisms / Organism Groups	No. of isolates at MIC (µg/mL; cumulative %) 2	4	8	16	32	64	128	256	MIC$_{50}$	MIC$_{90}$
Candida albicans (205)										
Pexiganan CLSI 24hr	0 (0.0%)	9 (8.9%)	2 (10.9%)	18 (28.7%)	70 (98.0%)	1 (99.0%)	1 (100.0%)		32	32
Pexiganan CLSI 48hr	0 (0.0%)	1 (1.0%)	1 (2.0%)	15 (16.8%)	46 (62.4%)	24 (86.1%)	14 (100.0%)		32	128
Pexiganan EUCAST			0 (0.0%)	1 (0.5%)	16 (8.3%)	58 (36.6%)	126 (98.0%)	4 (100.0%)	128	128

CLSI method read at 24hrs and at 48 hrs

Figure 1. Pexiganan activity against colistin resistant isolates of *A. baumannii-calcoaceticus* complex, *E. aerogenes*, *E. cloacae* complex and *K. pneumoniae*.



Conclusions

- Pexiganan was active against a broad range of microorganisms including those found in skin and skin structure infections. Activity was shown against MRSA, vancomycin resistant enterococci, β-hemolytic streptococci, carbapenem resistant Enterobacteriaceae, *P. aeruginosa* and *C. albicans*.
- With few exceptions, pexiganan exhibited potent *in vitro* antimicrobial activity against the Gram-positive, Gram-negative and fungal species/groups tested in this report.
- Pre-existing resistance to β-lactam agents, vancomycin, colistin or other drug classes did not significantly affect the activity of pexiganan against specific bacterial species/groups. *Morganella, Proteus* and *Serratia* spp. showed inherently higher MIC values.
- Pexiganan free base concentration in the current topical cream formulation in Phase 3 clinical development is 8000 µg/mL, which is orders of magnitude above the MICs for most organisms studied, indicating pexiganan levels are sufficient to eradicate most infecting organisms tested in this surveillance sample.
- Pexiganan is an important new therapeutic agent in the current environment of emerging multi-drug resistant pathogens.

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