

# The Arenicin-3 Derived Clinical Candidate AA139 Shows Potent Activity Against Gram-Negative Pathogens

**S. Neve\*, S. Lociuoro\*, I. Morrissey\*\*, S. Hawser\*\*, and P. Nordkild\*.**

\*Adenium Biotech, Ole Maaloesvej 3, DK-2200 Copenhagen, Denmark

\*\* IHMA Europe Sarl, 9A Route de la Corniche, CH-1066 Epalinges, Switzerland

Contact information  
sne@adeniumbiotech.com  
Phone +4560829303

## Revised Abstract

### Background

Arenicin-3 is an antimicrobial peptide isolated from *Arenicola marina* living on sediments in the tidal water of the North Sea. Extensive lead optimization of Arenicin -3 produced the clinical candidate AA139. This current study investigated the activity of AA139 against a recent worldwide collection of clinical isolates of *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Acinetobacter baumannii*

### Methods

Minimal inhibitory concentrations were determined using broth microdilution following CLSI guidelines (M7-A9). Recent clinical isolates of Gram-negative bacteria including multi-resistant *K. pneumoniae* (n=116), *P. aeruginosa* (n=111), *E. coli* (n=110) and *A. baumannii* (n=108) were investigated.

### Results

Summary MIC results are given in the Table. These results showed that AA139 exhibited potent antimicrobial activity against Gram-negative bacteria. Resistance to other agents did not appear to affect the activity of AA139.

Strain (No. of strains)	AA139 MIC (µg/ml)			
	MIC <sub>50</sub>	MIC <sub>90</sub>	MIN	MAX
<i>A. baumannii</i> (n=108)	2	2	0.5	4
<i>E. coli</i> (n=110)	0.5	0.5	0.25	2
<i>K. pneumoniae</i> (n=116)	2	4	0.5	8
<i>P. aeruginosa</i> (n=111)	4	8	1	16

### Conclusions

The clinical candidate AA139 showed a potent effect against a panel of important recently collected Gram-negative pathogens. This indicates that AA139 is a promising clinical candidate for the treatment of Gram-negative bacteria including those resistant to other antimicrobial agents.

## Background

The Arenicin family consists of three members: Arenicin-1 and -2, which were characterized by a Russian research group (Ovchinnikova et al., 2004), and Arenicin-3 which is a novel member of the family. Arenicin-3 was isolated from the marine lugworm *Arenicola marina*; it contains 21 natural amino acid residues constrained in an amphipathic beta hairpin structure by two disulfide bridges between Cys3, Cys20 and Cys7, Cys16. Four positively charged arginines, and 9 hydrophobic residues contribute to the amphipathic characteristics of the peptide.

In this study we present the Minimal Inhibitory Concentration of AA139 and comparators against a panel of world-wide recently collected Gram-negative clinical isolates.

## Susceptibility Testing

MIC tests were performed by broth microdilution against all isolates in line with Clinical and Laboratory Standards Institute (CLSI) susceptibility testing standards [1,2]. AA139 and polymyxin B were tested in the presence of 0.002% polysorbate 80 (present in test wells and during all dilution steps). Concurrent quality control testing using *Escherichia coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922 was performed as per CLSI M07-A9 [1]. CLSI breakpoints were used to determined susceptibility to comparator antimicrobials where available [2]. For the *Enterobacteriaceae*, polymyxin B susceptibility breakpoints for *P. aeruginosa* were used [2].

### Strain Statistics

	Year of collection			Source of isolated organism						
	2011	2012	2013	Blood	Bodily fluid	Gastro-intestinal	Genito-urinary	Intra-abdominal	Respiratory	Skin & skin structure
<i>A. baumannii</i>	0	55	53	-	16	16	14	-	46	16
<i>E. coli</i>	3	18	89	1	29	20	36	1	9	14
<i>K. pneumoniae</i>	0	41	75	-	29	20	45	-	14	8
<i>P. aeruginosa</i>	0	38	73	2	22	18	21	1	24	23
GRAND TOTAL	3	152	290	3	96	74	116	2	93	61

Geographic distribution was well-balanced with strains being equally distributed among Asia, North America, Europe and the rest of the world.

## References

1. CLSI, 2012. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard-Eighth Edition M07-A9. Clinical and Laboratory Standards Institute (CLSI), Wayne, PA 19087-1898 USA.
2. CLSI, 2014. Performance Standards for Antimicrobial Susceptibility Testing; Informational Supplement-Twenty-Second Edition M100-S24. Clinical and Laboratory Standards Institute (CLSI), Wayne, PA 19087-1898 USA.

## Results

**Table 1:** Minimal Inhibitory Concentrations (MIC) of AA139 and comparator antibiotics and % susceptibility according to CLSI breakpoints [2].

<i>A. baumannii</i> (n =108)						<i>E. coli</i> (n =110)					
	MIC <sub>50</sub>	MIC <sub>90</sub>	% S	% I	% R		MIC <sub>50</sub>	MIC <sub>90</sub>	% S	% I	% R
AA139	2	2	-	-	-	AA139	0.5	0.5	-	-	-
TZP	128	128	16.7	0.9	82.4	TZP	2	8	91.0	4.5	4.5
MEM	16	16	19.4	1.9	78.7	MEM	0.015	0.03	98.2	0	1.8
LVX	16	32	16.7	4.6	78.7	LVX	0.06	16	74.5	0	25.5
GEN	32	32	26.8	2.8	70.4	GEN	0.5	32	85.5	0.9	13.6
CAZ	32	32	19.4	1.9	78.7	CAZ	0.25	32	84.6	0.9	14.5
PMB	0.03	0.12	96.3	0	3.7	PMB	0.03	0.03	98.2	1.8	0
<i>P. aeruginosa</i> (n =111)						<i>K. pneumoniae</i> (n =116)					
	MIC <sub>50</sub>	MIC <sub>90</sub>	% S	% I	% R		MIC <sub>50</sub>	MIC <sub>90</sub>	% S	% I	% R
AA139	4	8	-	-	-	AA139	2	4	-	-	-
TZP	8	128	65.8	12.6	21.6	TZP	4	128	68.1	5.2	26.7
MEM	1	16	58.5	7.3	34.2	MEM	0.03	16	80.2	1.7	18.1
LVX	1	16	59.5	7.3	33.2	LVX	0.25	32	65.6	3.4	31.0
GEN	2	32	80.2	2.7	17.1	GEN	0.5	32	70.0	1.7	29.3
CAZ	4	32	70.3	5.4	24.3	CAZ	0.5	32	54.3	2.6	43.1
PMB	0.12	0.25	100	0	0	PMB	0.03	0.06	99.1	0.9	0

### Antimicrobial abbreviations:

TZP = piperacillin/tazobactam; MEM = meropenem; LVX = levofloxacin; GEN =gentamicin; CAZ =ceftazidime; PMB =polymyxin B

## Conclusion

- Percent of multidrug-resistant strains (resistance to  $\geq 3$  comparators) was particularly high for *A. baumannii* (82.4%), followed by *K. pneumoniae*, *P. aeruginosa* and *E. coli* with 31.0, 24.3 and 7.3%, respectively.
- Activity of AA139 was uniform among strains tested and there was no correlation with the geographical location or the source of isolation.
- Importantly, activity of AA139 was independent from strains' resistance profile

