

NZ17074 - A Novel Antimicrobial Peptide Showing Potent *In Vitro* Activity against Gram Negative Multi-resistant Clinical Isolates.

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Background:

Arenicin-3 is a beta-hairpin antimicrobial peptide isolated from the lugworm (*Arenicola marina*) living on sediments in the tidal water. NZ17074 is a derivative of Arenicin-3 which has then been chemically synthesised and purified. Please refer to Poster F1-2070 and F1-2072 for structure and mode of action.

Amino acid sequence for NZ17074: GFCWNVCVYRNGVRCRRRCN

This class of AMPs are known to exhibit cidal activities towards a diverse number of microorganisms. Susceptibility data on clinical isolates of *Klebsiella pneumoniae*, *Salmonella enterica*, *Pseudomonas aeruginosa* and *Escherichia coli* showed very potent activities. The purpose of the study was to investigate the activity profile of NZ17074 following standard CLSI conditions.

Methods:

MIC

Minimal inhibitory concentrations (MIC and MBC) were performed according to the general guidelines for susceptibility measurements using micro-broth dilution provided by CLSI/ NCCLS (M7-A5). The reference strains (*E. coli* ATCC 25922, *K. pneumoniae* ATCC700603 and *P. aeruginosa* ATCC 27853) were included as well as a number of control antibiotics.

Time-kill

All isolates were tested by a standard time-kill methodology as described by CLSI document M26-A with the exception of taking earlier time points (15, 30, 60, 120 and 180 minutes) than normal due to the rapid bactericidal nature of NZ17074. Multi-resistant clinical relevant isolates of *E. coli*, *K. pneumoniae*, *S. Typhimurium*, *P. aeruginosa* were tested in activity and time-kill experiments.

Activity in different pH and effect of Inoculum

Activity studies also included effect of bacterial inoculum and different pH growth conditions : MIC/MBC in triplicate for different species at pH 5.5, 6.0, 7.0 and 8.0.

Results:

MIC/ MBC

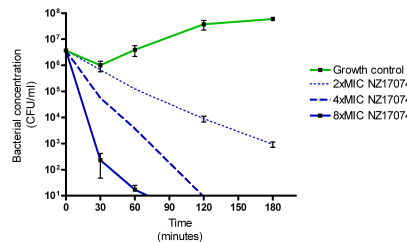
MIC results for different isolates of *Enterobacteriaceae* showed minor differences between the species (*E. coli* data shown below). Many different resistant isolates were included: ESBL positive stains, fluoroquinolone, aminoglycoside and carbapenem resistant strains.

MIC for NZ17074 (µg/ml)	0.125	0.25	0.5	1	2	4	8
<i>Escherichia coli</i> (n:45)	0	2	18	21	2	2	0

NZ17074 showed activity of 0.5-16 µg/ml for *P. aeruginosa* and non fermentors and the antimicrobial activity was found to be bactericidal (MBC~1-4xMIC),

Results for the time-kill experiments are shown in several figures on this poster. The effect of NZ17074 was shown to be concentration dependent causing a 3-log (99.9%) reduction in the viable bacteria population within 2 hours of NZ17074 exposure.

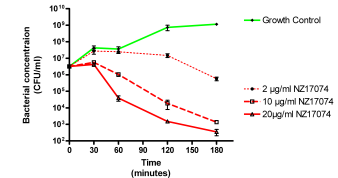
Time kill kinetic for #1536 Carb^R *Pseudomonas aeruginosa* (reference listed below)
(MIC for NZ17074: 4µg/ml). Mean and SEM of three CFU sampls.



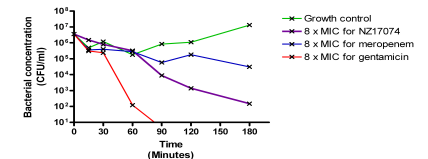
Kohlenberga et al. Outbreak of carbapenem-resistant *Pseudomonas aeruginosa* infection in a surgical intensive care unit. *Journal of Hospital Infection*. vol 74, (4) 2010, pp 350-357

Results:

Time kill experiments for different concentrations of NZ17074 for a ESBL positive *Escherichia coli* (AID#607) MIC: 1µg/ml. Mean bacterial concentration and SEM for 5 samples.



Timekill experiment for *Pseudomonas aeruginosa* ATCC27853
(MIC for NZ17074: 4µg/ml)



The inoculum had only minor influence (increase in MIC value of four fold) on the MIC efficacy when inoculum CFU was raised from 10^2 to 10^7 CFU/ml . Results for different pHs in the growth media showed only minor influence the antimicrobial activity (range of a two- four fold decrease of MICs from pH 6.0 to 8.0).

Conclusions for NZ17074:

- * Active against multiresistant *Enterobacteriaceae* (MIC₉₀ ~ 1µg/ml for *E.coli*)
- * Bactericidal (3-log reduction within few hours)