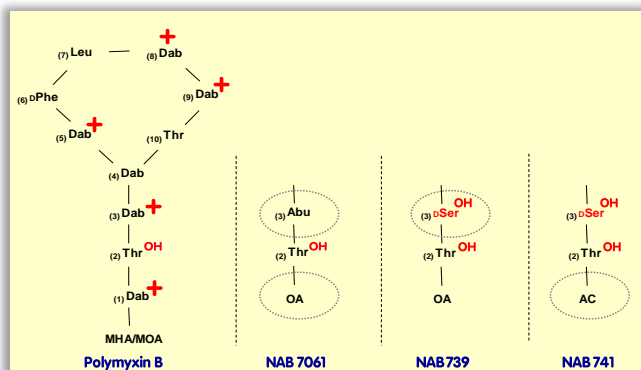


## Novel polymyxin derivatives for the treatment of serious Gram-negative hospital infections



The need for well-tolerated antibiotics that are active against extremely multiresistant (XMR) Gram-negative bacteria is urgent. Today the most problematic Gram-negative bacterium is *Pseudomonas aeruginosa*. However, enteric Gram-negatives and *Acinetobacter* are responsible for more than 80% of all the hospital infections caused by Gram-negative bacteria, and now they are rapidly becoming resistant to most antibiotics that are currently used to treat them (Livermore, 2009, J Antimicrob Chemother 64: Suppl. 1, i29).

Plasmid-mediated carbapenemases, transferred from *Klebsiella pneumoniae*, have now been found in *Escherichia coli*, the clinically most important species of Gram-negative enteric bacteria. Also plasmid-mediated methylases that cause resistance to all aminoglycosides have been encountered (Doi *et al.*, 2008, Antimicrob Agents Chemother 52:2287-2288). Finally and quite alarmingly, a single genetic element conferring transferable resistance to carbapenems, aminoglycosides and fluoroquinolones has been reported in *K. pneumoniae* (Rice *et al.*, 2008, Antimicrob Agents Chemother 52:3427-3429).

One can predict that when eventually the most problematic bug has changed from *Pseudomonas* through *K. pneumoniae* to *E. coli*, also the use of antibiotics has become much more species-specific than what it is today, facilitated by rapid PCR and microarray-based in vitro diagnostic assays.

The emergence of XMR Gram-negative bacteria has necessitated the use of polymyxins (polymyxin B and colistin) as the agents of last resort despite their known nephrotoxicity (for a review, see Nation and Li, 2009, Curr Opin Infect Dis 22:535-543; Li *et al.*, 2006, Lancet Infect Dis 6:589-601).

Northern Antibiotics Ltd., a Finnish biotech company, has developed highly active polymyxin derivatives with significantly lower potential to cause nephrotoxicity.

The derivatives contain three (3) positive charges only, while polymyxin B and colistin have five (5). They bind to the isolated brush-border membrane of rat kidney at an affinity which is only 1/7-1/5 of that for polymyxin B and about 1/3-1/2 of that for gentamicin (Hiroshima University, Japan).

More than forty derivatives have been synthesized and they fall into two groups that differ in their mode of action (Vaara *et al.*, 2008, Antimicrob Agents Chemother 52:3229-3236). The lead compound of the first group, NAB739, acts directly against enteric Gram-negative bacteria and *A. baumannii*, whereas NAB7061 and NAB741, the lead compounds of the second group, sensitize target bacteria to other antibiotics such as rifampin and clarithromycin.

Highlights of the antibacterial properties of the novel polymyxin derivatives include:

- NAB739 has a MIC<sub>90</sub> of 1 µg/mL and the MIC range of 0.5 – 2 µg/mL for twenty (20) *E. coli* strains, including three (3) ESBL strains and three (3) strains that are ESBL plus carbapenemase producers. For polymyxin-susceptible strains of *K. pneumoniae* (n =11), including six (6) carbapenemase producers, the MIC<sub>50</sub> is 2 µg/mL and the MIC range is 1-4 µg/mL. For *Enterobacter cloacae*, *Klebsiella oxytoca*, and *Citrobacter freundii* (n = 8), the MIC is 2 µg/mL.
- While the MIC of NAB739 for *A. baumannii* (3 strains) is 2-8 µg/mL, as low a concentration as 0.5 µg/mL reduces the MIC of rifampin from 4 µg/mL to 0.05 µg/mL (*A. baumannii* ATCC 19606). Furthermore, the MIC of clarithromycin is reduced from 8 µg/mL to 0.5 µg/mL, and the MIC of vancomycin from 256 µg/mL to 3 µg/mL. The use of polymyxin derivatives in combination with another antibiotic may have the additional advantage of reducing the risk of resistance development, as already suggested for colistin by Li *et al.* (Clin Inf Dis 2007;45:594-598).
- NAB7061, at a concentration of 4 µg/mL, reduces the MIC of clarithromycin for *K. pneumoniae* ATCC 13883 from 12 µg/mL to 0.19 µg/mL and the MIC of rifampin from 16->32 µg/mL to 0.064 – 0.125 µg/mL. For *E. coli* and *E. cloacae*, the sensitization factors are even higher.
- The antibacterial properties of NAB741 are very similar to those of NAB7061.

The efficacy of both NAB739 and NAB7061 has been verified using an experimental *E. coli* peritonitis model in mice (Statens Serum Institut, Copenhagen, Denmark; Vingsbo-Lundberg *et al.*, abstract FI-3966, 48th Interscience Conference on Antimicrobial Agents and Chemotherapy, Washington D.C., September 2008).

- A reduction of 4.8 log<sub>10</sub>, 4.5 log<sub>10</sub>, and 5.3 log<sub>10</sub> in the bacterial loads compared to saline control was achieved at 4 h after the completed treatment with NAB739 at a dose of 1 mg/kg, 2 mg/kg, and 4 mg/kg, respectively, administered twice at an interval of 2 h.
- Also the combination treatment with NAB7061 (5 mg/kg) and bacteriostatic erythromycin (10 mg/kg) resulted in reduced bacterial loads. At 1 h after the completed treatment (4 hours post infection), a 2.0 log<sub>10</sub> CFU reduction (p<0.05) was observed and the CFU levels remained low during the subsequent three hours.
- Together with the ability to reduce bacterial loads, the NAB compounds also improved the clinical status of the mice.

Parameter	NAB 7061	NAB 739	NAB 741	Colistin
Half-life (min)	66.2 ± 12.3	69.0 ± 21.9	32.7 ± 2.41	74.6 ± 13.2
Volume of distribution (ml/kg)	339 ± 96	222 ± 20.5	243 ± 24.0	496 ± 60
Clearance (ml/min/kg)	3.84 ± 0.75	2.63 ± 0.54	7.39 ± 0.85	5.2 ± 0.4
Urinary recovery (% of dose)	7.16 ± 3.70	19.4 ± 7.38	50.9 ± 13.6	0.18 ± 0.14
Renal clearance (ml/min/kg)	0.28 ± 0.16	0.53 ± 0.30	3.78 ± 1.11	0.010 ± 0.008

<sup>1</sup> mean ± SD

In the pharmacokinetic studies in rats (Monash University, Melbourne, Victoria, Australia), the urinary recovery and renal clearance of the NAB compounds were markedly higher than those of colistin (Ali *et al.*, Journal of Antimicrobial Chemotherapy doi:10.1093/jac/dkp331).

Since only the linear side chain of the polymyxin molecule has to be modified, the semi-synthetic production of the NAB compounds is believed to be simple. The methods are well-known, and polymyxin itself is a very inexpensive raw material.

The novel polymyxin derivatives are covered worldwide by two patent applications, as follows.

- Polymyxin derivatives and uses thereof - WO 2008/017734 A1, US-2008-0287345-A1 and US-2009-0215677-A1
- Short fatty acid tail polymyxin derivatives and uses thereof - WO 2009/098357 A1, US-2009-0239792-A1.