Antibiofilm effects of exogenous nitric oxide against *Klebsiella pneumoniae*

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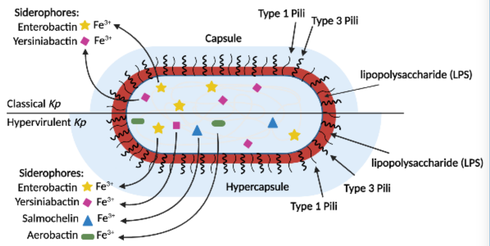
INTRODUCTION

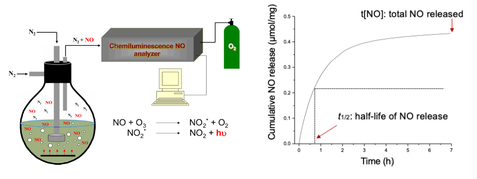
Klebsiella pneumoniae is an opportunistic bacterium that is found naturally in the gastrointestinal tract of humans and animals.[1] *K. pneumoniae* makes up for one-third of Gram-negative infections including pneumonia, urinary tract infection, and liver abscesses.[1,2] *K. pneumoniae* biofilms are mostly found on medical devices including urinary catheters.[5] Increasing antibiotic resistant *K. pneumoniae* strains have emerged, with the most recent strain exhibiting resistance to last-resort antibiotics such as carbapenems.[3] Of the seven *K. pneumoniae* strains studied in this paper, four strains are classical, four are hypervirulent, with one displaying hypermucoviscosity. Classical strains have higher resistance and usually affect those who are immunosuppressed, while hypervirulent strains have a more severe pathology and can affect immunocompetent. The biofilm consists of a matrix containing proteins, polysaccharides, and DNA effective penetration by antibiotics.[5] The four virulent factors associated with *K. pneumoniae* are pili (type 1 and type 3), capsule, lipopolysaccharide (LPS), and iron carriers termed siderophores.[4] Biofilm formation is dependent on type 3 pili which help with adhesion and compound the virulence of *K. pneumoniae*.[4] Nitric oxide (NO) is an endogenously produced free radical that plays a role in the innate immune response.[6] Nitric oxide is a promising antibacterial agent due to its ability to be involved in nitrosative and oxidative stress.[7] Nitric oxide has been shown to effectively destroy biofilms where antibiotics have been found ineffective.[7] The aim of this work is to optimize biofilm growth procedures for seven strains of strains of *K. pneumoniae* and examine how NO affects these biofilms.



KLEBSIELLA PNEUMONIAE STRAINS USED

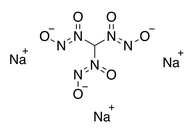
Strain	Type	Capsule
MPK103	cKp	Normal
ATCC 13883	cKp	Normal
NTUH-K2044	hvKp	Hyper
MGH 78578	cKp	Normal
KPPR1S	hvKp	Hyper
KPPR1-Δ*rmpD*	hvKp	Hyper
KPPR1-Δ*wcaJ*	hvKp	None

NOA Analysis

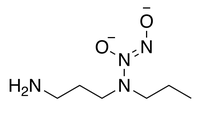


	t[NO] (umol/mg)	$T_{1/2}$ (h)
PAPA/NO	9.4 ± 0.1	0.4 ± 0.02
MD3	5.9 ± 0.4	5.9 ± 0.7

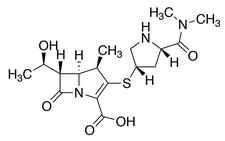
Treatment Compounds Investigated:



MD3



PAPA/NO



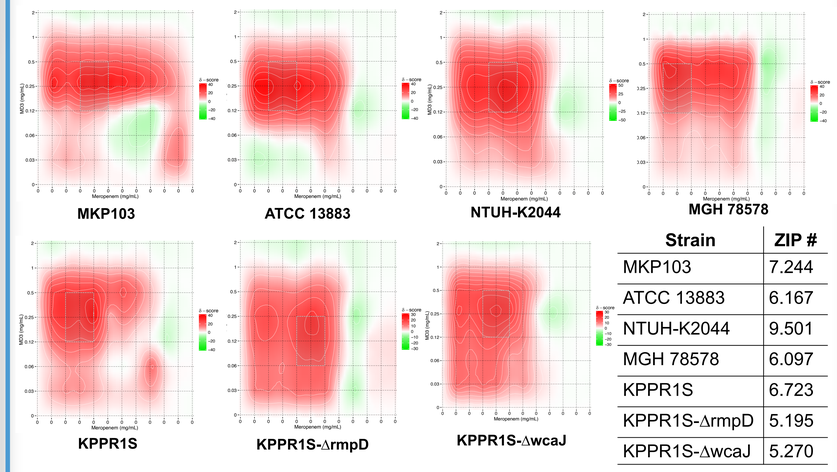
Meropenem

ANTIBACTERIAL ASSAYS OF NO-RELEASING MOLECULES

Strain	MD3		PAPA/NO	
	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)
MKP103	0.125	0.125-0.25	1-2	2
ATCC 13883	0.125	0.125-0.25	1-2	2
NTUH-K2044	0.125	0.125-0.25	2	2
MGH 78578	0.125	0.125	1-2	2
KPPRIS	0.125	0.125-0.25	2	2
KRRPIS- ΔrmpD	0.125	0.25	2	2-4
KPPRIS-ΔwcaJ	0.125	0.125-0.25	2	2

- For each NO-releasing molecule, eradication of all seven strains was achieved at similar doses
- *C*-diazeniumdolate (MD3) eradicates *K. pneumoniae* at lower does than *N*-diazeniumdiolates (PAPA/NO)

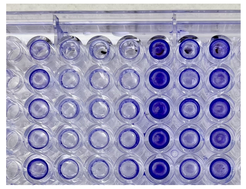
SYNERGY ASSAY



Strain	ZIP #
MKP103	7.244
ATCC 13883	6.167
NTUH-K2044	9.501
MGH 78578	6.097
KPPR1S	6.723
KPPR1S-ΔrmpD	5.195
KPPR1S-ΔwcaJ	5.270

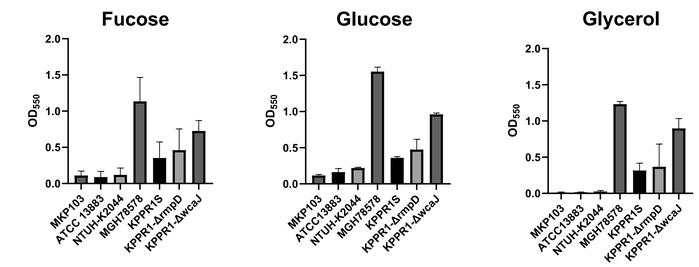
- All have zero interaction potency (ZIP) synergy scores to be less than 10, showing that MD3 and meropenem when used in combination have additive effects
- Areas on the graph in dark red show the optimal concentrations for MD3 and meropenem to be additive

FORMATION OF *KLEBSIELLA PNEUMONIAE* BIOFILMS



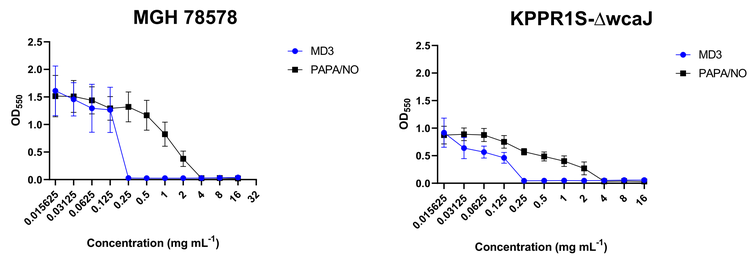
- Formation of biofilms
  - M63 media
  - Incubate for 24 hours at 37˚C anaerobically
  - Cells are rinsed with water
- Quantification of biofilms
  - Biofilms adhere to the bottom of the well
  - Crystal violet staining analyzed at 550 nm
- Bacteria with no capsule formation, exhibited more robust biofilm formation while those with hyper capsule exhibited very little biofilm formation

OPTIMIZATION OF CARBON SOURCE FOR BIOFILM GROWTH



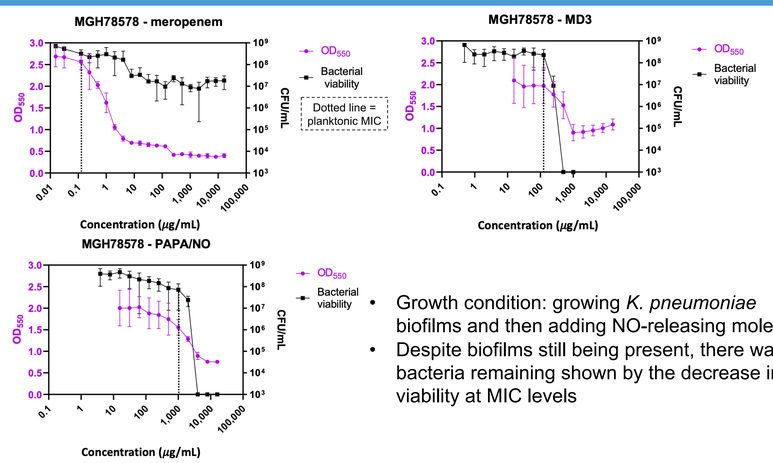
- Analyzed biofilms grown in M63 media using different carbon sources
- In comparison to fucose and glycerol, glucose formed the most robust biofilms

NITRIC OXIDE PREVENTS BIOFILM FORMATION



- Growth condition: growing *K. pneumoniae* with NO-releasing molecule
- Biofilms were able to be eradicated at MIC levels when grown in combination with the NO-releasing molecule

NITRIC OXIDE DISPERSES ESTABLISHED BIOFILMS



- Growth condition: growing *K. pneumoniae* biofilms and then adding NO-releasing molecule
- Despite biofilms still being present, there was no bacteria remaining shown by the decrease in viability at MIC levels

CONCLUSIONS

- Glucose was chosen as the main carbon source for biofilm formation
- NO-releasing molecules were able to eradicate and penetrate the biofilm
- The synergistic relationship between MD3 and meropenem is additive

FUTURE DIRECTIONS

- Investigate the relationship between meropenem and other NO-releasing small molecules including PAPA/NO, DPTA/NO, and DETA/NO
- Determine if biofilms act differently in anerobic or aerobic conditions
- Collect MIC/MBC data on other *N*-diazeniumdiolates with the seven strains of *K. pneumoniae*
- Test biofilm adhesion on other surfaces including glass and metal

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