Patogeni respiratori difficili: il ruolo di NAC

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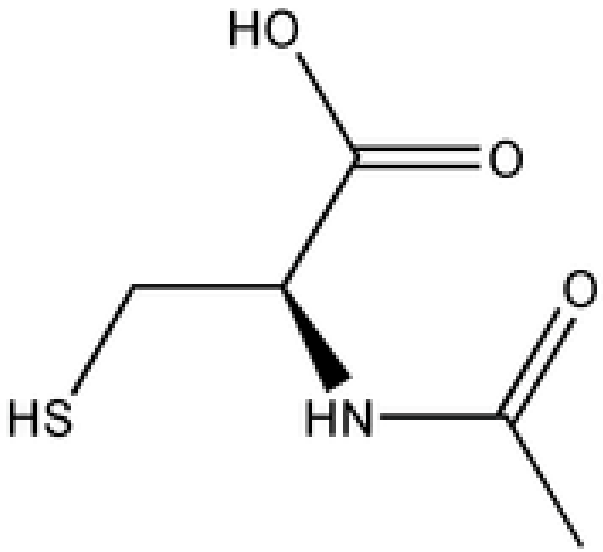
Disclosure

Consultancies, Research grants, Travel grants from: Zambon

N-Acetylcysteine (NAC)

- **Mucolytic agent with antioxidant and anti-inflammatory properties:**
  - disruption of disulfide cross-bridges in the glycoprotein matrix of mucus
  - precursor of glutathione
  - reducing agent

IV
oral
inhalation
direct instillation



N-AcetylCysteine

N-Acetylcysteine (NAC)

- **Mucolytic agent with antioxidant and anti-inflammatory properties:**
  - disruption of disulfide cross-bridges in the glycoprotein matrix of mucus
  - precursor of glutathione
  - reducing agent
- **Antidote for paracetamol intoxication (since 1970s)**
- **Commonly used for the management of chronic respiratory disorders, such as CF, COPD, and bronchiectasis (since early 1960s)**

Blasi – Resp Med 2016
Samuni – BBA 2013

NAC and bacterial infections

- **Negative interaction with the activity of antibiotics?**
  (conflicting data reported since 1970s)

Parry - AAC 1975
Goswami – AAC 2010

NAC and bacterial infections

NAC, even at the high concentration likely achievable by topical administration, does not inhibit the activity of antibiotics, with the notable exception of carbapenems

against a Large Collection of Respiratory Pathogens

Giulia Landini,[a] Tiziana Di Maggio,[a] Francesco Sergio,[b] Jean-Denis Docquier,[a] Gian Maria Rossolini,[a,c,d,e] Lucia Pallecchi[a]

Landini – AAC 2016
Rodriguez-Beltran – AAC 2015

NAC and bacterial infections

- **Negative interaction with the activity of antibiotics?**
  (conflicting data reported since 1970s)

- **Intrinsic antimicrobial activity?**

Antimicrobial activity of NAC

- **first reported in late 1970s**
- **NAC can affect growth of several Gram-negative and Gram-positive species (including anaerobes and mycobacteria), and yeasts**

Pseudomonas spp.
Acinetobacter baumannii,
Escherichia coli
Klebsiella pneumoniae
Enterobacter cloacae
Proteus spp.
Haemophilus influenzae

Staphylococcus spp.
Streptococcus spp.,
Enterococcus faecalis
Corynebacterium sp.

Mycobacteria (incl. *M. tuberculosis*)

Candida albicans

Prevotella intermedia
Helicobacter pylori
Propionibacterium acnes
Actinomyces naeslundii
Lactobacillus salivarius

Amaral - BMC Micro 2017
Domenech - AAC 2017
Jang - J Microbiol 201
Eroshenko - Microb Pat 2017
Volgers - FEMS Micr Lett 201
Moon - J Microbiol 2016
Ferris – JCM 2016

Blasi - Resp Med 2016 and refs therein

Antimicrobial activity of NAC

- **first reported in late 1970s**
- **NAC can affect growth of several Gram-negative and Gram-positive species (including anaerobes and mycobacteria), and yeasts**
- **concentrations achievable by topical administration**

Blasi - Resp Med 2016 and refs therein

Amaral - BMC Micro 2017
Domenech - AAC 2017
Jang - J Microbiol 201
Eroshenko - Microb Pat 2017
Volgers - FEMS Micr Lett 201
Moon - J Microbiol 2016
Ferris – JCM 2016

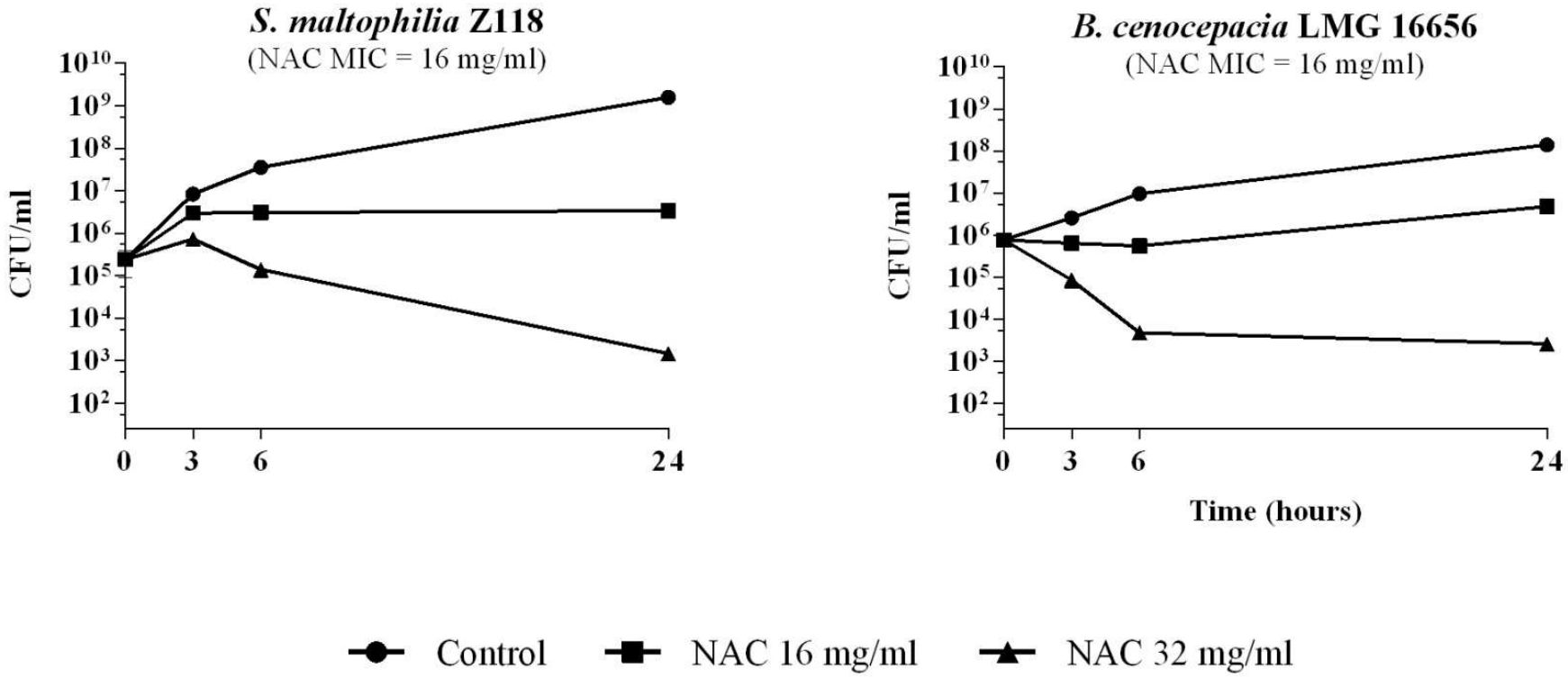
Antimicrobial activity of NAC

Stenotrophomonas maltophilia
Burkholderia cepacia complex

- ✓ *S. maltophilia* (n = 19) and BCC (n = 19), including CF isolates
- ✓ NAC MIC ranged 16-32 mg/ml (MBC from 32 to > 32 mg/ml)
- ✓ Sub-MIC concentrations (i.e., 0.25 × MIC) slowed down the growth kinetics of most strains
- ✓ In some cases, killing activity observed at 2 × MIC

Antimicrobial activity of NAC

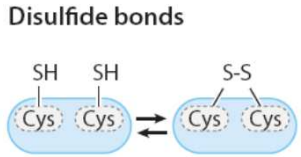
Stenotrophomonas maltophilia
Burkholderia cepacia complex

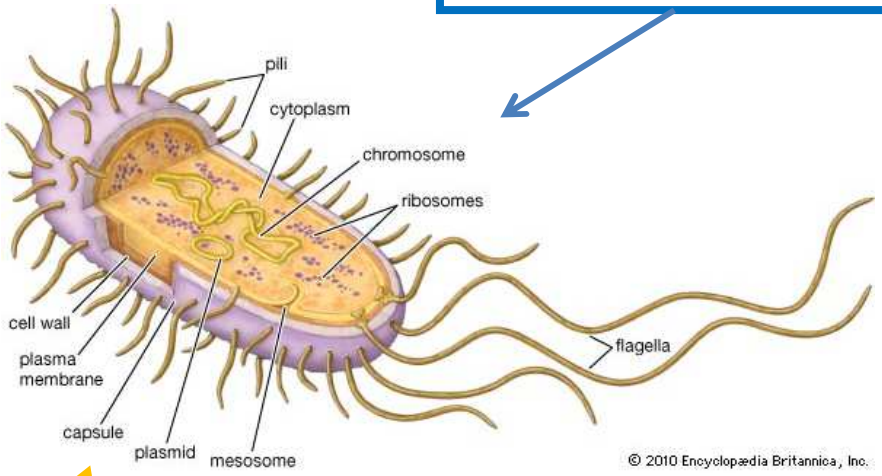


Mechanisms hypothesized for NAC antimicrobial activity

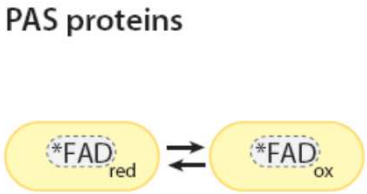
Competitive inhibition of cysteine utilization

Reduction of disulfide bonds in bacterial proteins





Modulation of intracellular signal transduction pathways (e.g. reduction of PAS-domains in "sensor" proteins)



Blasi - Resp Med 2016

Sporer - Ann Rev Biochem 2017

NAC and bacterial infections

- **Negative interaction with the activity of antibiotics?**
  (conflicting data reported since 1970s)

- **Intrinsic antimicrobial activity?**

- **Antibiofilm activity?**

Antibiofilm activity of NAC

- **First reported in late 1990s**
- **Detected with biofilms of various species (even mixed biofilms)**

Pseudomonas spp.
Acinetobacter baumannii,
Escherichia coli
Klebsiella pneumoniae
Enterobacter cloacae
Proteus spp.
Haemophilus influenzae

Staphylococcus spp.
Streptococcus spp.,
Enterococcus faecalis
Corynebacterium sp.

Rapidly growing mycobacteria

Candida albicans

Prevotella intermedia
Propionibacterium acnes
Actinomyces naeslundii
Lactobacillus salivarius

Blasi – Resp Med 2016 and refs therein

Domenech - AAC 2017
Eroshenko - Microb Pat 2017
Ferris - JCM 2016
Moon - J Microbiol 2016
Munoz-Egea - MDR 2015

Antibiofilm activity of NAC

- **First reported in late 1990s**
- **Detected with biofilms of various species (even mixed biofilms)**
- **NAC anti-biofilm activity by:**
  - Inhibition of biofilm formation
  - Disruption of preformed biofilms

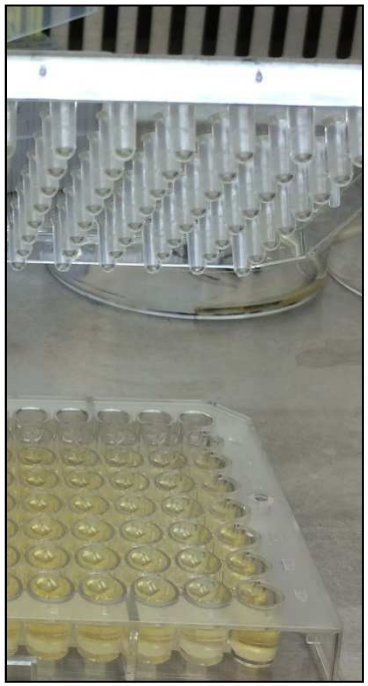
Blasi – Resp Med 2016 and refs therein

Domenech - AAC 2017
Eroshenko - Microb Pat 2017
Ferris - JCM 2016
Moon - J Microbiol 2016
Munoz-Egea - MDR 2015

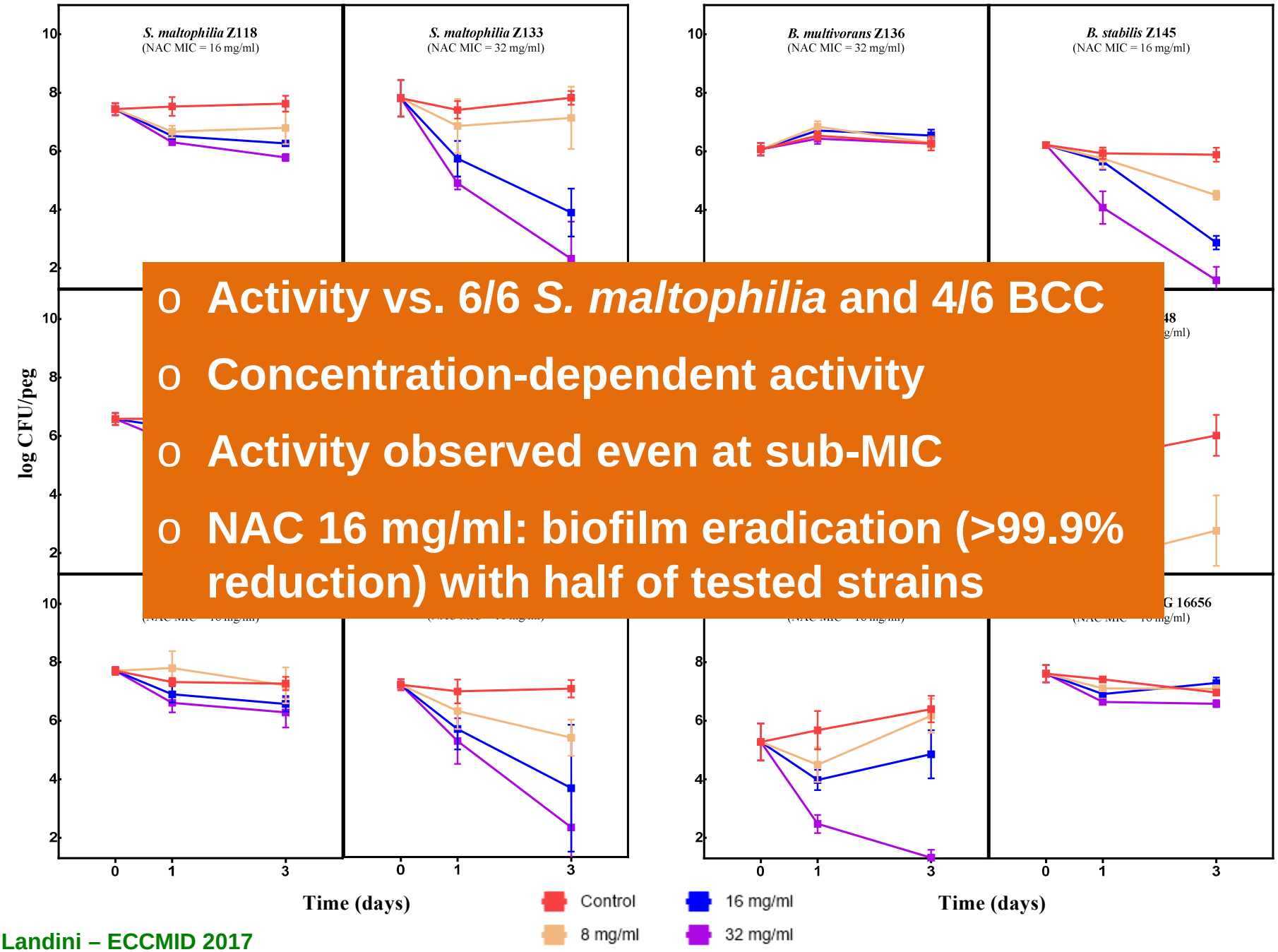
Antibiofilm activity of NAC

Stenotrophomonas maltophilia
Burkholderia cepacia complex

- Time-kill assays with preformed biofilms (2-days old) exposed to high NAC concentrations



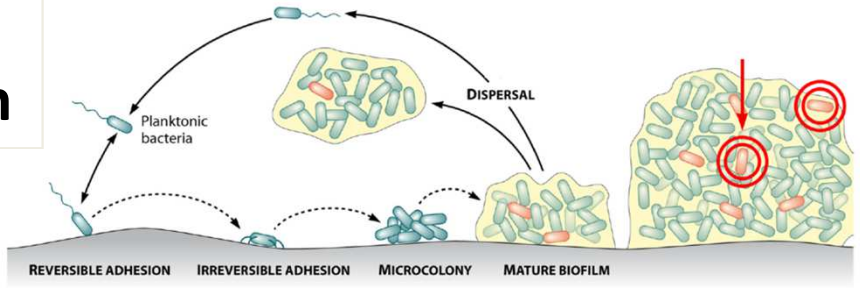
MBEC Assay
Biofilm grown on microtiter peg lids



- Activity vs. 6/6 *S. maltophilia* and 4/6 BCC
- Concentration-dependent activity
- Activity observed even at sub-MIC
- NAC 16 mg/ml: biofilm eradication (>99.9% reduction) with half of tested strains

Mechanisms hypothesized for NAC antibiofilm activity

Inhibition of biofilm formation



Disgregation of biofilm

- Impact on cell physiology?
- Interaction with biofilm matrix?

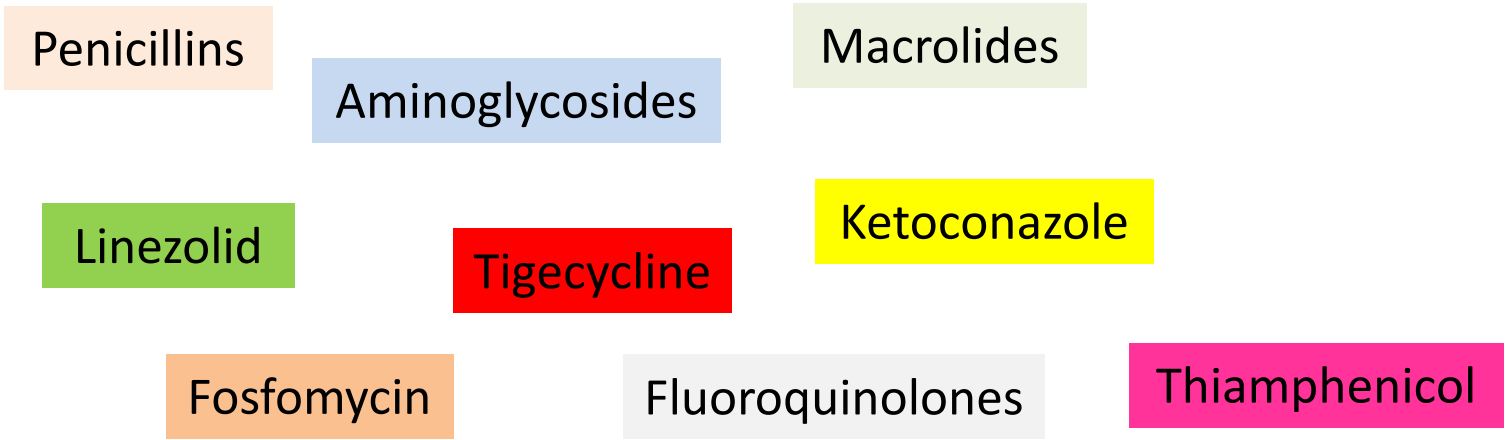
Blasi - Resp Med 2016

NAC and bacterial infections

- **Negative interaction with the activity of antibiotics?**
  (conflicting data reported since 1970s)
- **Intrinsic antimicrobial activity?**
- **Antibiofilm activity?**
- **Synergism with antibiotics?**

Synergism of NAC/antibiotic combinations

- **conflicting data reported since late 1970s**
- **observed either with planktonic and biofilm cultures**



- **No data with COLISTIN**

Muñoz-Egea - MDR 2015
El-Baky – AJIDM 2014
Mansouri - AAC 2013
Leite - EIMC 2013
Abd El-Aziz – IJAA 2012
Del Prado - DMID 2010
Venkatesh - JMM 2009
Aslam - AAC 2007
Marchese - IJAA 2003
Bozzolasco - GIMMOC 2002

Blasi – Resp Med 2016 and refs therein

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Review

Inhaled colistin monotherapy for respiratory tract infections in adults without cystic fibrosis: a systematic review and meta-analysis

Konstantinos Z. Vardakas [1,2], Georgios L. Voulgaris [1,3], George Samonis [4], Matthew E. Falagas [1,2,4,5,*]

- **High local concentrations**
- **Reduced systemic toxic effect**
- **Alone or in combination with IV?**
- **CF, VAP, non-CF bronchiectasis**

Colistin *vs* some relevant respiratory pathogens:

Pseudomonas aeruginosa	Rare R, resistance phenotype instable, but….
Acinetobacter baumannii	Sporadic R, resistance phenotype instable, but….
Stenotrophomonas maltophilia	No clinical breakpoints, high R rates
Burkholderia cepacia complex	Naturally resistant (> L-Ara4N in LPS)
Achromobacter xylosoxydans	No clinical breakpoints, high R rates

- **Reports of *in vivo* emergence of "stable" colistin-R strains**
- **Reports of clonal outbreaks of colistin-R strains in CF**
- **Large-scale dissemination of stable colistin-R clones**

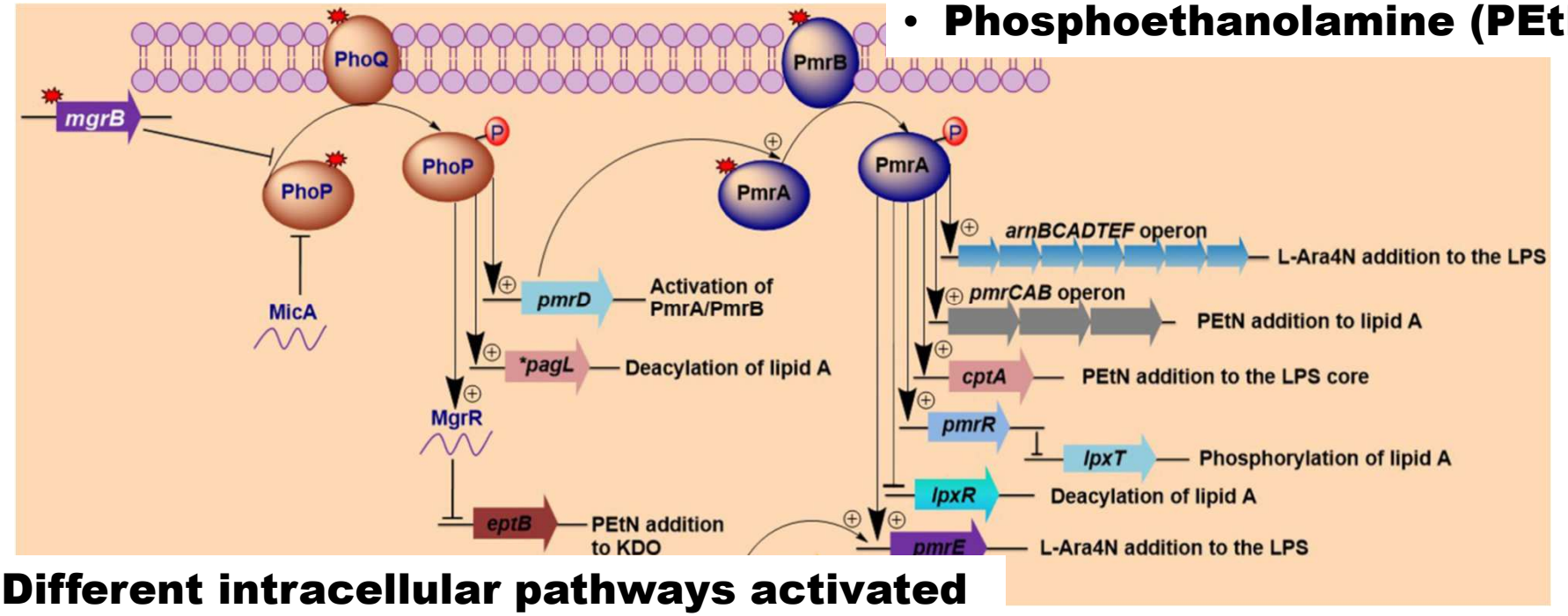
Durante-Mangoni – DMID 2015
Krogh – JCF 2008
Nowak – JAC 2017

Poirel – CMR 2017
Lee – SR 2016
Abbott – SRCCM 2015
Olaitan – FM 2014

The most common mechanisms of colistin resistance are modifications to the LPS (i.e. cationic substitutions of the phosphate groups), which decrease the net negative charge of LPS, thus reducing colistin/LPS interaction

Most common substitutions:

- **4-amino-4-deoxy-L-arabinose(L-Ara4N)**
- **Phosphoethanolamine (PEtN)**



Different intracellular pathways activated by a great variety of environmental stimuli (e.g. colistin exposure, but also pH, pO2, escape from immune response etc...)

Olaitan - FM 2014
Poirel - CMR 2017
Needham - RNM2013

Colistin/NAC combinations against difficult respiratory pathogens: planktonic cultures

- **Relevant synergism of colistin/NAC combinations against Col-R *A. baumannii* and *S. maltophilia***

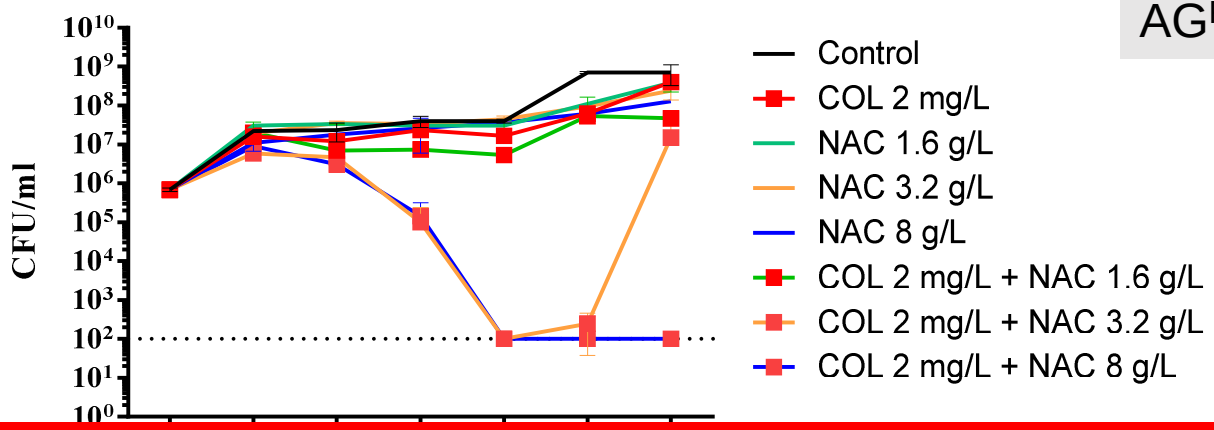
✓ E.g. NAC 8 mg/ml lowered the MIC of colistin to 2 µg/ml for all tested Col-R strains (*A. baumannii*, n = 7; *S. maltophilia*, n = 18)

✓ No synergism observed with Col-S strains (*A. baumannii*, n = 9; *S. maltophilia*, n= 2)

BAL, CB^R, FQ^R, AG^R, SXT^R, CL^R

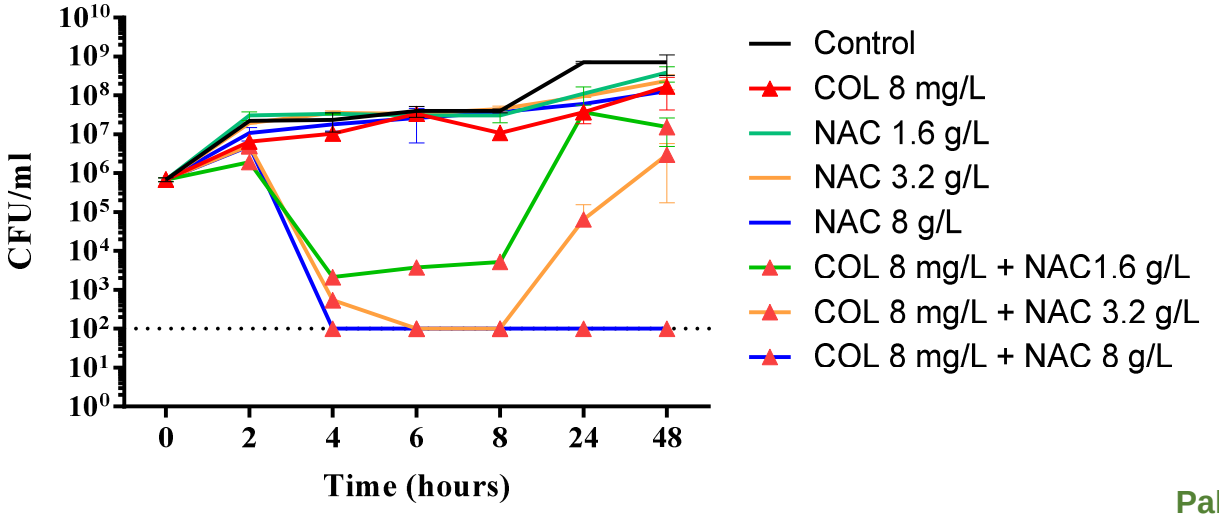
***A. baumannii* Z165**

(COL MIC = 32 mg/L; NAC MIC = 32.000 mg/L)



Dose-dependent potentiaton of COL activity

Similar results with other Col-R *A. baumannii* and *S. maltophilia* strains, including CF strains



Pallecchi - ECCMID 2017

Pallecchi - ERS Congress 2017

Colistin/NAC combinations against difficult respiratory pathogens: planktonic cultures

- Relevant synergism of colistin/NAC combinations against "Col-R" *A. baumannii* and *S. maltophilia*

- **Relevant synergism observed also against an *in vitro* selected Col-R mutants of *P. aeruginosa*, while no effect observed with Col-S *P. aeruginosa strains* (n = 10)**

- **Synergism observed also with a Col-R *Achromobacter xylosoxidans* from CF (n = 1)**

Pallecchi - unpublished

Colistin/NAC combinations against difficult respiratory pathogens: biofilms

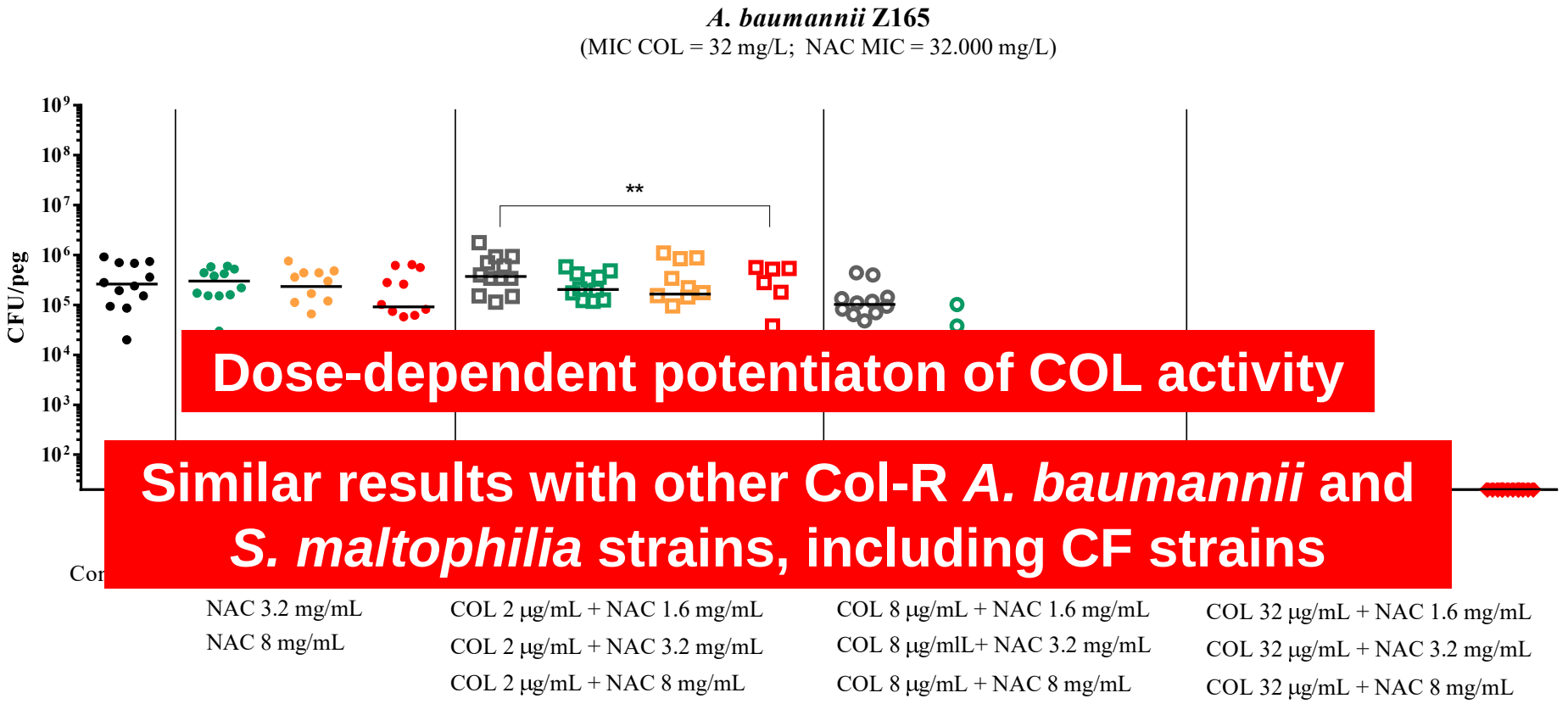
- **Relevant synergist effect against Col-R *A. baumannii* and *S. maltophilia* strains**

MBEC Assay
Biofilm grown on microtiter peg lids

Pallecchi - ECCMID 2017
Pallecchi - ERS Congress 2017

7 days growth
24 hours challenge

BAL, CB^R, FQ^R, AG^R, SXT^R, CL^R



** $p < 0.01$ (two-tailed unpaired Student's t-test)

Colistin/NAC combinations against difficult respiratory pathogens: biofilms

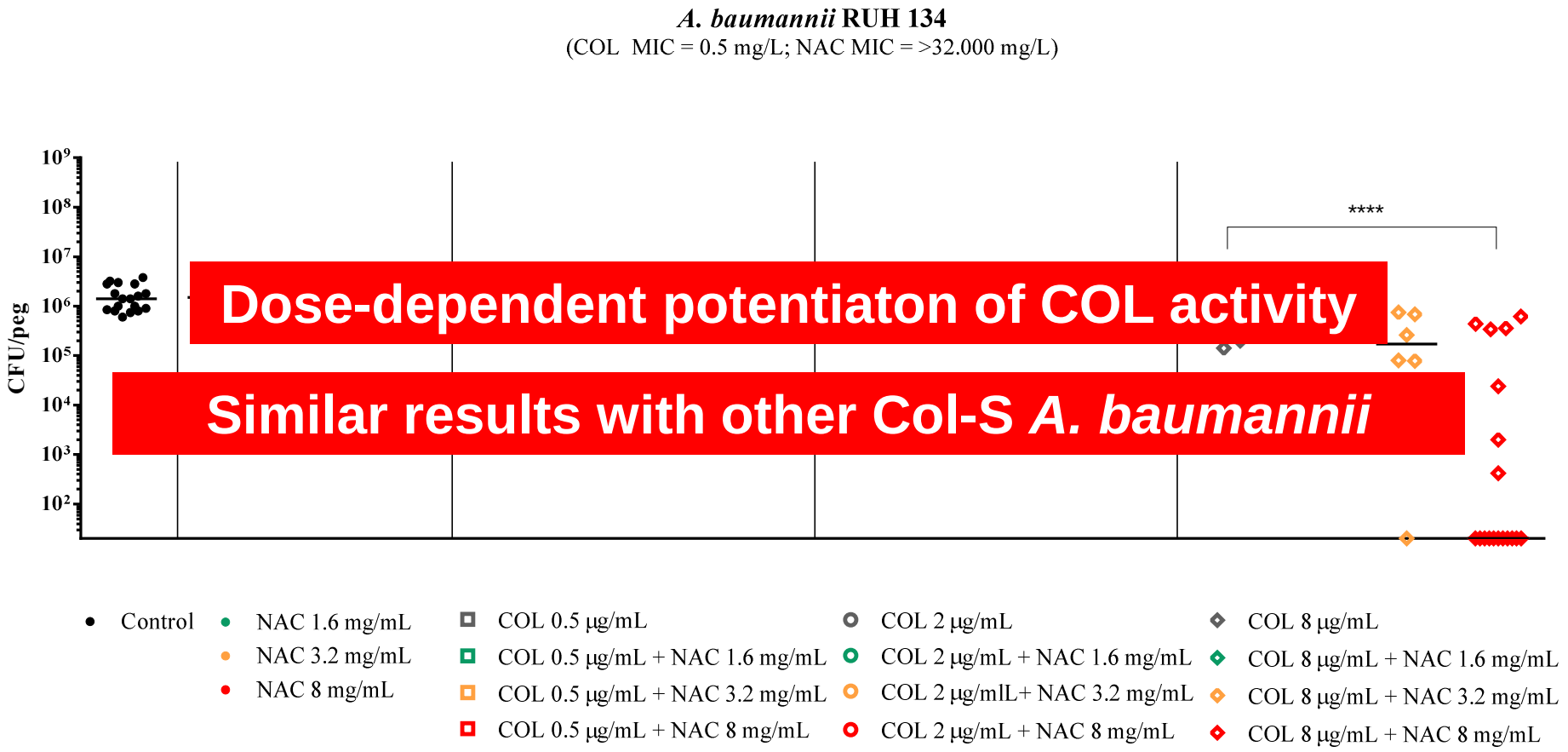
- Relevant synergist effect against "Col-R" *A. baumannii* and *S. maltophilia* strains

- **Some synergism observed also with Col-S *A. baumanni* and *P. aeruginosa* strains**

Pallecchi - unpublished

7 days growth
24 hours challenge

Reference strain for Global Clone 2, AG^R, SXT^R

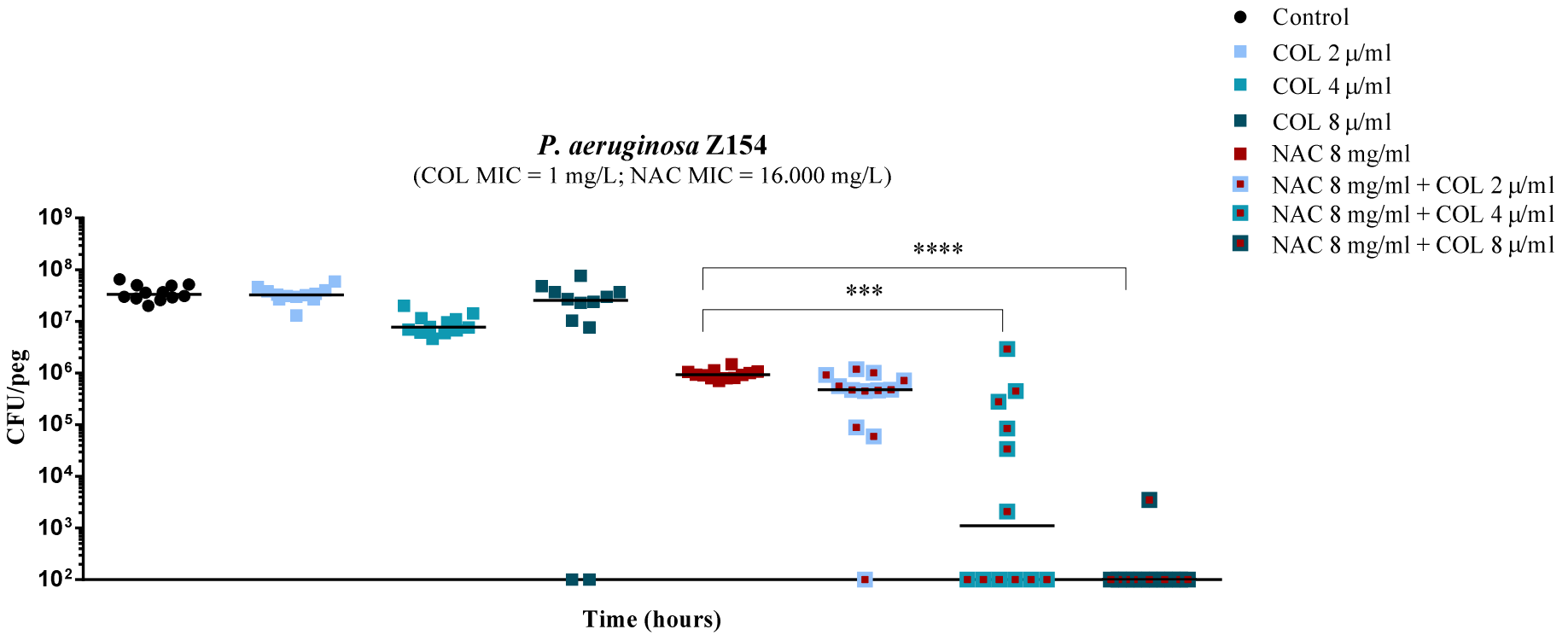


**** $p < 0.0001$ (two-tailed unpaired Student's t-test)

Pallechi - unpublished

2 days growth
24 hours challenge

Col-S mucoid strain from CF



*** p < 0.001, **** p< 0.0001 (two-tailed unpaired Student's t-test)

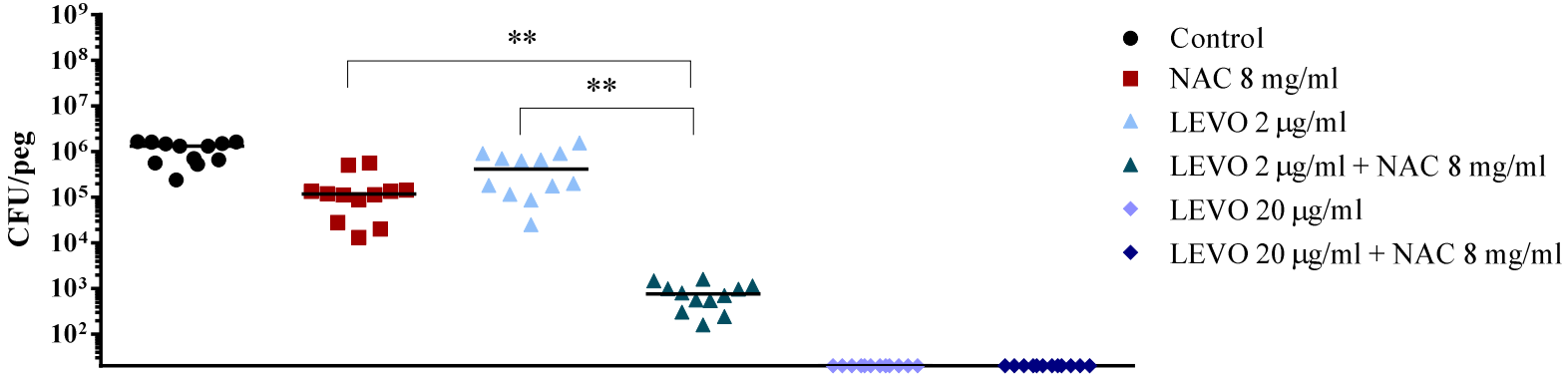
Fosfomycin or Levofloxacin/NAC combinations against CF *P. aeruginosa*: biofilms

- **Antibiofilm synergism detected with two CF strains (to be further investigated)**

Pallecchi - unpublished

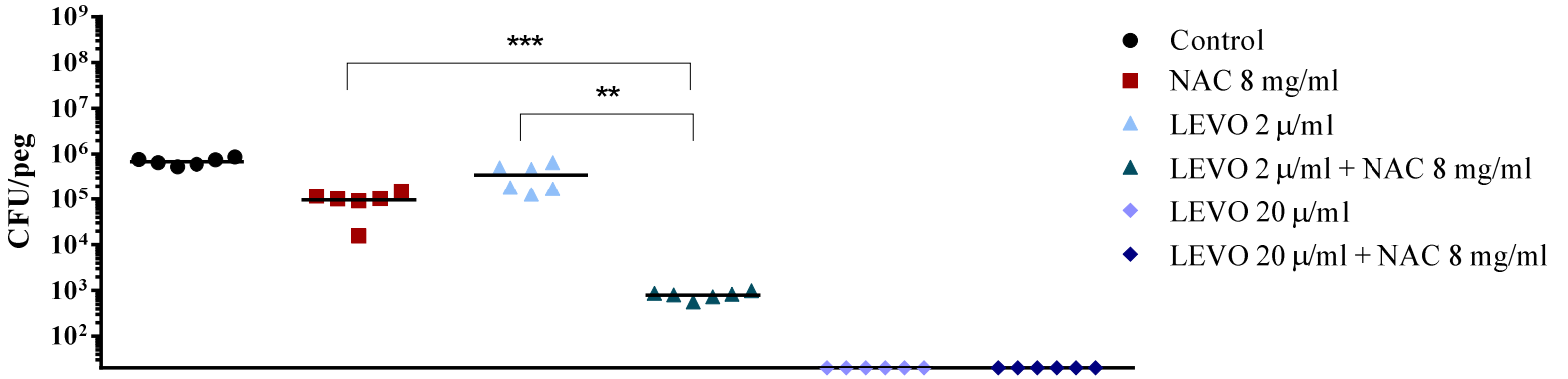
***P. aeruginosa* Z33**

(LEVO MIC = 2 mg/L; MIC NAC = >16.000 mg/L)



***P. aeruginosa* Z35**

(LEVO MIC = 2 mg/L; NAC MIC = 16.000 mg/L)

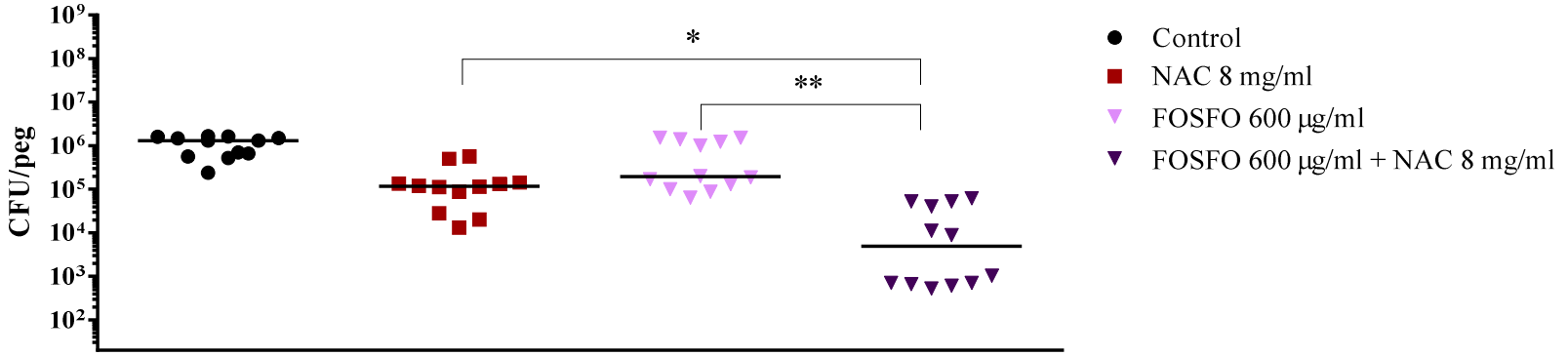


* p< 0.5; ** p< 0.01, *** p < 0.001 (two-tailed unpaired Student's t-test)

Pallechi – unpublished

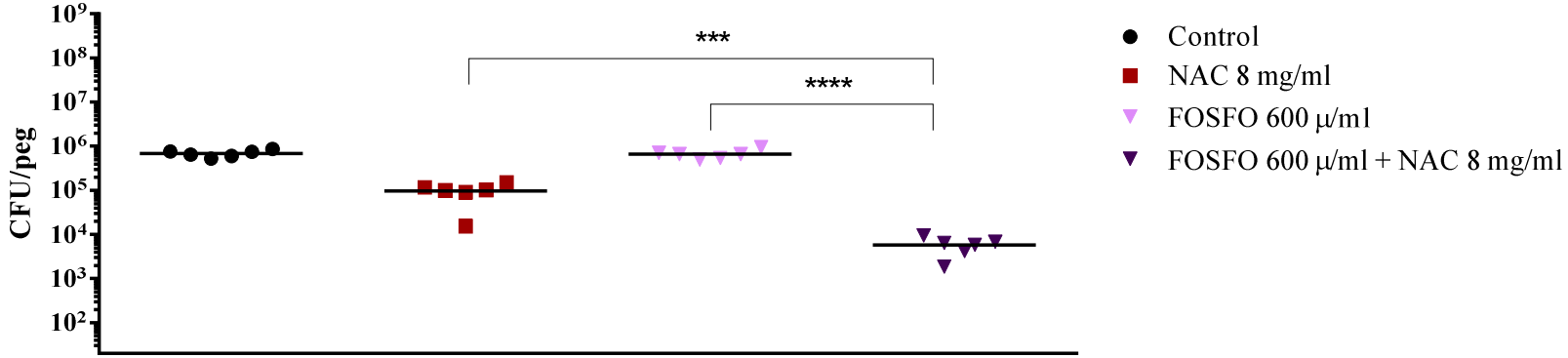
***P. aeruginosa* Z33**

(LEVO MIC = 2 mg/L; MIC NAC = >16.000 mg/L)



***P. aeruginosa* Z35**

(LEVO MIC = 2 mg/L; NAC MIC = 16.000 mg/L)



* p< 0.5; ** p< 0.01, *** p < 0.001, **** p < 0.0001 (two-tailed unpaired Student's t-test)

Pallechi – unpublished

Conclusions (1)

- NAC (even at high concentrations) does not negatively affect antibiotic activity, except for carbapenems

- High NAC concentrations achievable via topical administration may exert antimicrobial and antibiofilm activity against several respiratory pathogens, including “difficult” pathogens (e.g. *S. maltophilia* and *B. cepacia* complex)

- NAC negatively affects common mechanisms of colistin resistance, and this seems a general phenomenon (i.e. observed with *S. maltophilia*, *A. baumannii*, *A. xylosoxidans*, and *P. aeruginosa*)

Conclusions (2)

- NAC/colistin combinations achievable via topical administration exhibit an synergistic antibiofilm activity against *S. maltophilia, A. baumannii* and *P. aeruginosa*

- NAC/fosfomycin and NAC/levofloxacin combinations achievable via topical administration might exhibit synergistic antibiofilm activity against *P. aeruginosa* from CF (to be further investigated)