EPITHELIX
In vitro respiratory solutions

In vitro human airway epithelial platform for the development of novel anti-bacterial drugs

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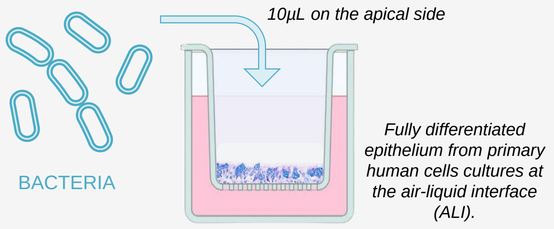
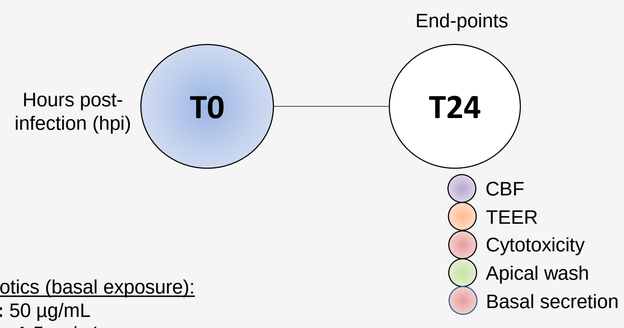
Respiratory bacterial infections cause frequently mild to severe diseases worldwide. To develop new anti-bacterial drugs more predictive research tools are needed. We report herein the use of 3D epithelia made of primary human airway epithelial cells, MucilAir™, for anti-bacterial drug screening. As proof-of-concept, typical disease-causing bacteria is used to infect human nasal epithelia reconstituted from a pool of 14 donors.

Tested bacteria *Pseudomonas aeruginosa* (Pa), *Staphylococcus aureus* (Sa), *Acinetobacter baumannii* (Ab), *Streptococcus pneumoniae* (Sp), *Haemophilus influenzae* (Hi)

Inhibitor of bacterial growth used Meropenem (Mero), Amoxicillin (Amox)
Positive controls Cytomix for inflammation, Triton X-100 for cytotoxicity
Negative controls Vehicle (VH)

Methods



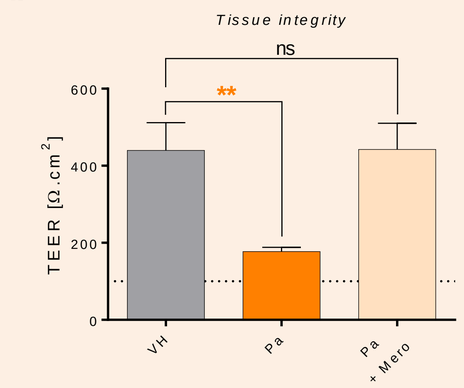


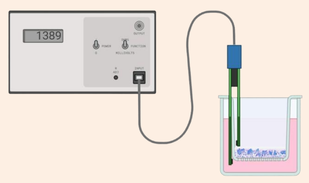
Inoculum used:
Pa: 30 CFU/MucilAir™ – PAO1WT – N=6
Sa: 10^2 CFU/MucilAir™ – ATCC 29213 (MSSA) – N=3
Ab: 30 CFU/MucilAir™ – CIP7010 – N=3
Sp: 10^4 CFU/MucilAir™ – SP19F – N=6
Hi: 10^4 CFU/MucilAir™ – ATCC 49247 – N=3

Antibiotics (basal exposure):
Mero: 50 µg/mL
Amox: 1.5 µg/mL

TEER measurement for tissue integrity (EVOM™)

A.





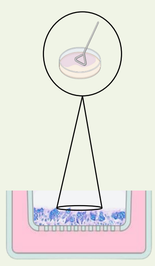
B.

***Staphylococcus aureus* (Sa)**	↓ at 24 hpi *
+ Meropenem	= vehicle level
***Acinetobacter baumannii* (Ab)**	↓ at 24 hpi **
+ Meropenem	= vehicle level
***Streptococcus pneumoniae* (Sp)**	No effect
+ Amoxicillin	= vehicle level
***Haemophilus Influenzae* (Hi)**	No effect
+ Meropenem	= vehicle level

Figure 1. Transepithelial electrical resistance (TEER) measurement. TEER was assessed at 24 hours post infection. TEER threshold is 100Ω.cm². **A.** MucilAir™ Pool-Nasal was infected with Pa (PAO1WT). Vehicle control was compared to infected condition and infected MucilAir™ Pool-Nasal treated appropriate antibiotic (mean±SEM). **B.** Summary of TEER data for Epithelix strains. Student's t test (Prism 6.0 GraphPad, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, ns: not significant).

End-points from apical Wash

Colony-forming unit (CFU)



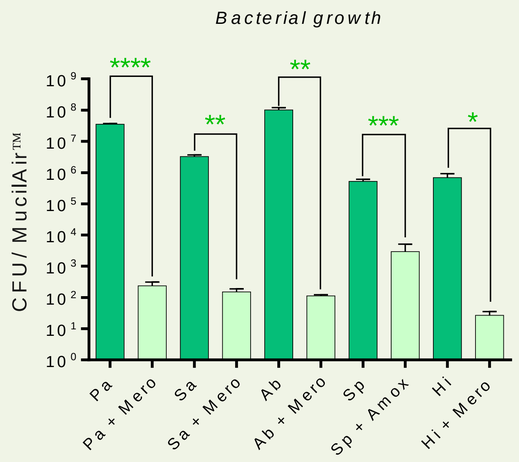
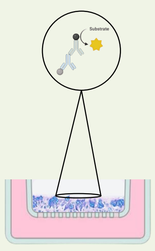


Figure 3. Apical growth of bacteria with or without appropriate antibiotics on MucilAir™ Pool-Nasal. Tissues were infected with Pa, Sa, Ab, Sp or HI. Enumeration of bacteria at the apical side was assessed at 24 hours post-infection on specific agar plates. Infected condition was compared to infected MucilAir™ Pool-Nasal treated with appropriate antibiotic (mean±SEM). Student's t test (Prism 6.0 GraphPad, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001).

Mucin secretion



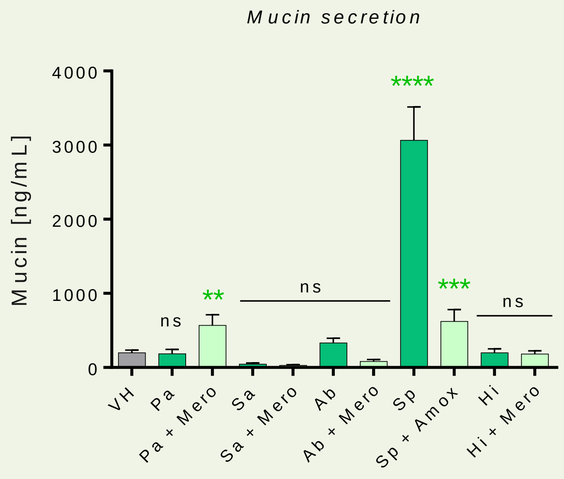


Figure 4. Effect of bacterial strains on mucin secretion. Apical washes was sample at 24 hours post-infection. Vehicle control was compared to infected conditions with or without the appropriate antibiotic (mean±SEM). Student's t test (Prism 6.0 GraphPad, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, ns: not significant).

Microscopy for cilia activity (Cilia-X)

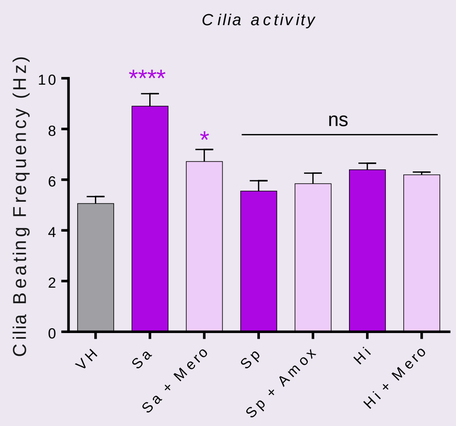
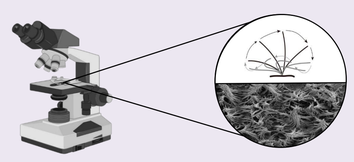
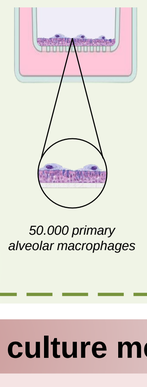


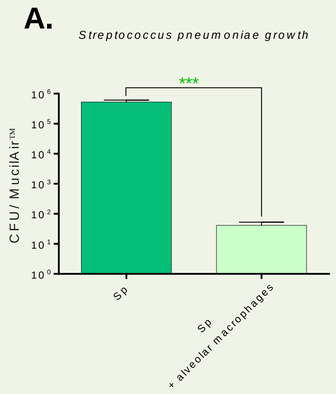
Figure 2. Effect of bacterial strains on Cilia Beating Frequency (CBF). CBF measurement was assessed at 24 hours post-infection. Vehicle control was compared to infected conditions with or without the appropriate antibiotic (mean±SEM). Student's t test (Prism 6.0 GraphPad, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, ns: not significant).



Alveolar macrophages effect



A.



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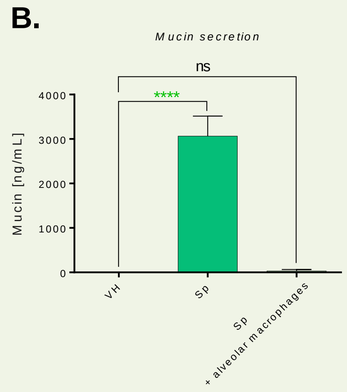
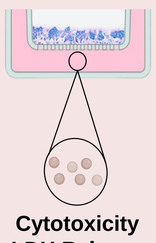


Figure 5. Effect of alveolar macrophages on Sp infection. **A.** Sp growth at 24 hours post-infection. Infected condition was compared to infected tissues treated with alveolar macrophages. **B.** Mucin secretion 24 hours post-infection. Vehicle control was compared to infected condition and infected condition treated with alveolar macrophages. (mean±SEM). Student's t test (Prism 6.0 GraphPad, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, ns: not significant).

End-points from basal culture medium

Cytotoxicity LDH Release



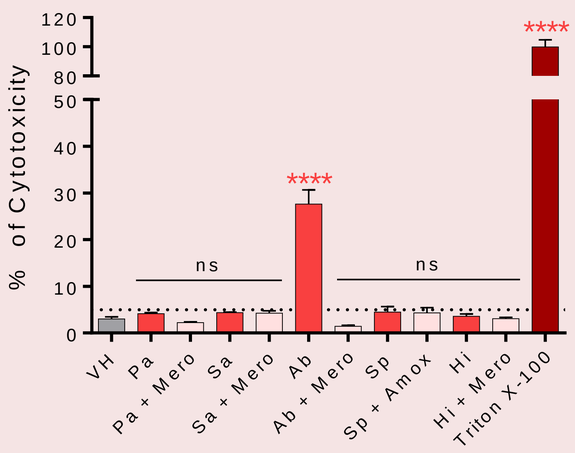
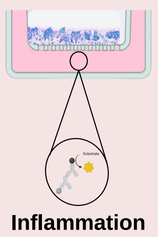


Figure 6. Effect of bacterial strains on Lactate deshydrogenase (LDH) release. LDH release was measured 24 hours post-infection. LDH threshold is 5%. Vehicle control was compared to infected conditions with or without the appropriate antibiotic and Triton X100 control (mean±SEM). Student's t test (Prism 6.0 GraphPad, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, ns: not significant).

Inflammation Il-8 secretion



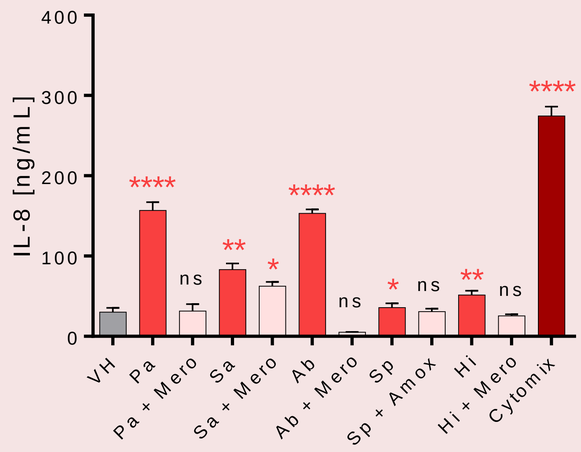


Figure 7. Effect of bacterial strains on Interleukin 8 (IL-8) release. Basal media for IL-8 analysis was sample 24 hours post-infection. Vehicle control was compared to infected conditions with or without the appropriate antibiotic and Cytomix control (mean±SEM). Student's t test (Prism 6.0 GraphPad, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, ns: not significant).

CONCLUSION

Fingerprint of bacterial-specific effects on standardized *in vitro* nasal epithelium (MucilAir™ Pool-Nasal) are herein reported.
Antibiotics like Meropenem inhibits the bacterial growth and abrogates its side effects. Similarly, macrophages in co-culture decrease the growth of Sp and prevent the bacterium-induced increase of mucin secretion. Additionnal end-point can be used such as mucociliary clearance (MCC), intratissular bacterial localisation, etc… These results suggest that MucilAir™ is a reliable tool for anti-bacterial drug development.