Investigating the role of non-typeable *Haemophilus influenzae*, in cystic fibrosis lung infection

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URSS UNDERGRADUATE RESEARCH SUPPORT SCHEME

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Introduction

A sticky situation: Cystic Fibrosis

- In UK, CF affects more than **10,900 individuals**[1]
- **Autosomal recessive** disorder commonly caused by **F508-del mutations** in **CFTR** anion channel.
- **F508-del: protein-misfolding**[2]**;** proteins cannot be completely glycosylated & is prematurely degraded in the proteasome, preventing Cl^- ion conduction in the extracellular membranes.
- **Consequence:** Accumulation of thick viscoelastic, sticky mucus blocking CF airways providing an ideal niche for microbes to grow[3].

NTHi Biofilms: A Possible Link to Early CF Lung Disease

1) **NTHi → understudied opportunistic pathogen** that colonises CF lungs.
2) **First pathogens** to invade CF lungs, with increased prevalence to **~30%** within **10 years**[7].
3) Potentially, NTHi infection may **affect colonisations** of severe, later-stage pathogens, such as ***P. aeruginosa***[8]**.**
4) NTHi **AMR increased** ~30% in 15 years, with **52%** found to be resistant to one or more antibiotics[9].

Aim: Use **ex-vivo pig lung model**, with **synthetic CF sputum media** (SCFM) to answer key questions about NTHi in CF lungs.

What is NTHi?

- **Gram-negative**, pleomorphic opportunistic, coccobacilli
- Grows both **aerobically** requires presence of **X (hemin)** & **V NAD** factors and **anaerobically**[4]
- Grown on **chocolate agar** under lab conditions
- **non-encapsulated polysaccharides**[5]
- **Nasopharynx:** key reservoir of NTHI infections from which the bacterium may spread to the lower respiratory tract[6]

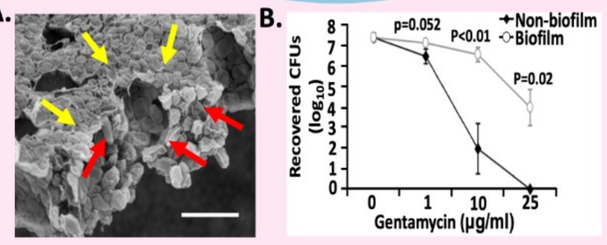
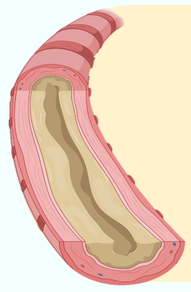


Figure 1. Evidence of NTHi biofilm formation during CF infection by SEM and effects of biofilm formation on antibiotic resistivity[10].

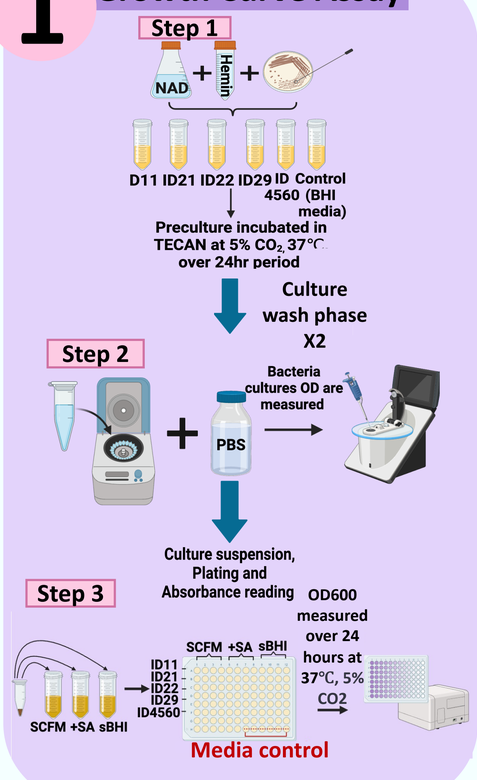


Key Research Questions:

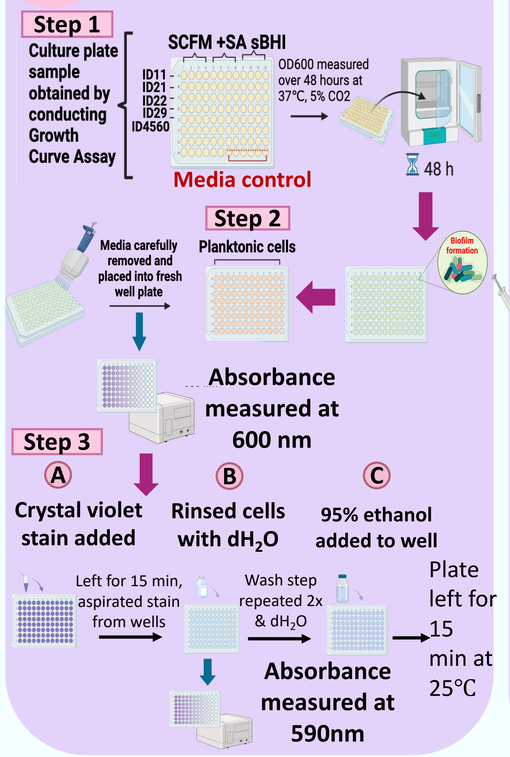
1) Can non-typeable *H. influenzae* form biofilms?
2) Are they more tolerant to antibiotics?
3) Does antibiotic treatment lead to resistance or increased susceptibility to other pathogens?
4) Are there any other approaches to test for NTHi growth in the ex-vivo pig lung model?

Methodology

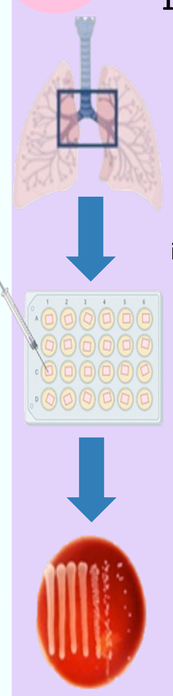
1 Growth Curve Assay



2 Biofilm Assay



3 CFU Assay



1. Bronchiole tubes are cut, washed using **ampicillin** and **ciprofloxacin** antibiotics to remove endogenous bacteria and cut into square pieces
2. Squares are infected with **NTHi** and grown at **37 ℃** for **48 hours** in **SCFM** media.
3. **Total Colony Forming Units (CFU)** are calculated

Results

Supplemented SCFM2 promotes non–typeable *H. influenzae* 'biofilms' *in vitro*?

24-hour Growth Curve of NTHi in different media conditions

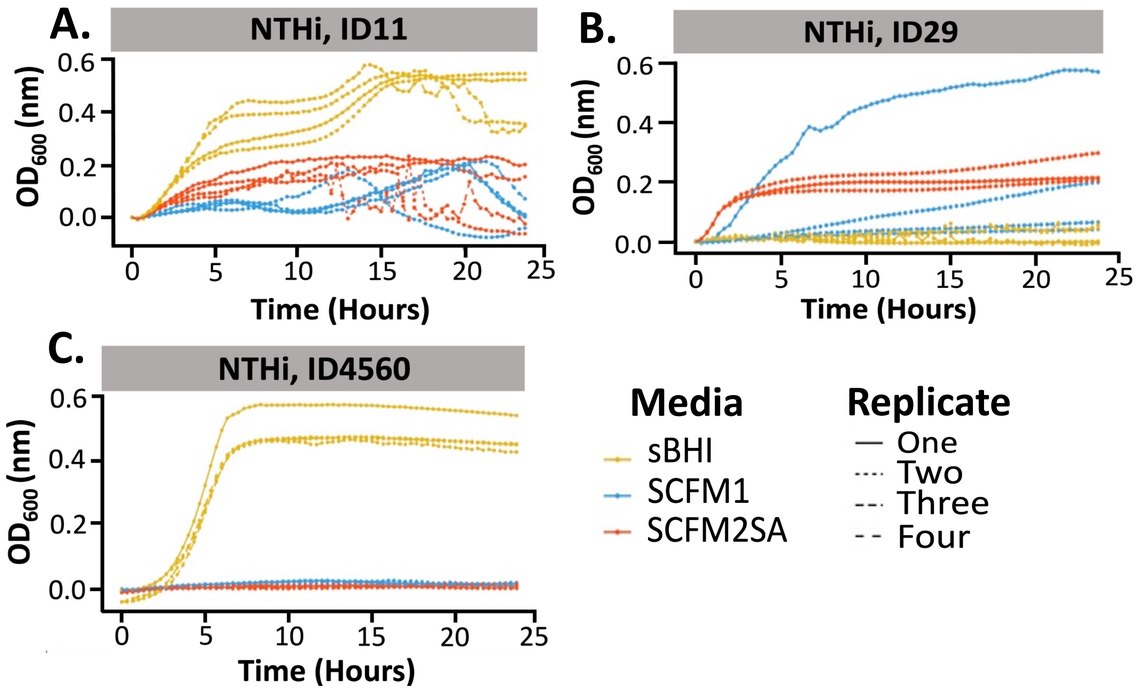


Figure 2. NTHi growth rate in different media conditions. Isolates ID11 and ID4560 have shown to grow effectively on sBHI media compared to other media types. However, isolate ID29 does not grow well on sBHI but effective growth is determined in SCFM1 media. All isolates have average growth in SCFM2SA.

Relative Biofilm allocation of NTHi in different media conditions

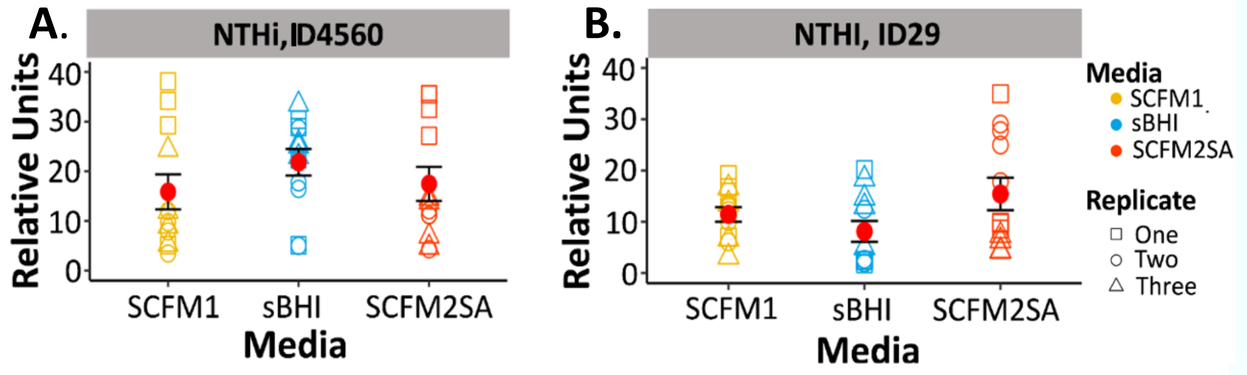


Figure 3. Static growth of NTHi for 48 hours followed by crystal violet staining. (A) Two-way ANOVA revealed no statistically sig. difference in media type for ID4560 (Clinical strain) relative biofilm formation **F(2,31) = 0.923, p = 0.912. (B)** However, there was a statistical sig. difference in media type for ID29 (Laboratory strain) relative biofilm formation, **F(2,31) = 8.684, p = 0.001****.

Conclusions

- ✓ **All NTHi** isolates manifest **variable growth rates** in the **media** conditions tested.
- ✓ **SCFM2SA** have shown to be the **most effective** media for all NTHi isolate growth, proving that the addition of **sialic acid** to **SCFM1** promotes **successful** bacterial growth.
- ✓ **ID29** (Lab strain) showed a sig. difference in **Biofilm formation**, whereas **ID4560** (Clinical strain) showed no sig. difference. The sig. differences in **Biofilm formation**, indicate that **biofilms** could impact **CF pathogenesis**.
- ✓ **Ampicillin** antibiotics is **most effective** against **ID21** & **ID4560 (MIC = 4 µg/ml)**. However, it is **least effective** against **ID11 & ID29** with **ID11** being the most **resistant (MIC = > 128 µg/ml)**

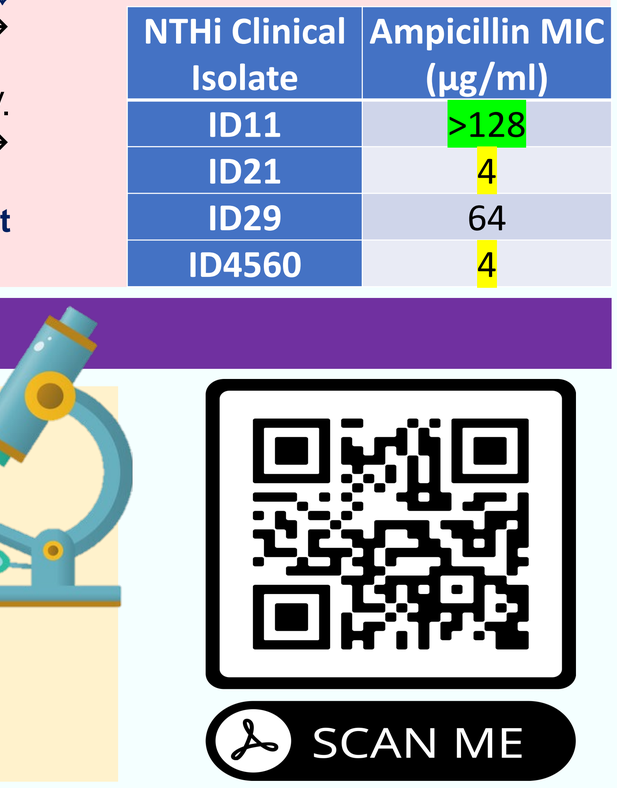
NTHi Antibiotic Susceptibility Test: MIC

- **Minimum inhibitory concentration (MIC):** Lowest antibiotic concentration that prevents visible bacterial growth after overnight incubation with media.
- **Pink: Metabolically active** (live bacteria)
- **Blue: Not-metabolically active** (non-growing bacteria)

Analysis

EUCAST MIC breakpoints[11]: **S ≤ 1, R > 1**
Result: Clinical Isolates **MIC** is **> 1** ∴ R

- ➢ ID21 & ID4560 MIC = **4 µg/ml** (lowest) ∴ ↓ Amp dose required to inhibit growth → **effective**
- ➢ ID11 & ID29 MIC = >128 & 64 µg/ml (V. high) ∴ ↑ dose required to inhibit growth → **ineffective**
- ➢ ID11 MIC = >128 µg/ml → most **resistant** strain, could be pathogenic



NTHi Clinical Isolate	Ampicillin MIC (µg/ml)
ID11	>128
ID21	4
ID29	64
ID4560	4

Discussion

Future Research

1) **Confocal microscopy** & **SEM/TEM** imaging of biofilms, and **transcriptome analysis** of NTHi with **RNAseq**[12]
2) Test AMR with **different** antibiotics to **compare** whether the **resistivity patterns** are **similar/different** to **ampicillin**
3) **Virulence assays**: determine **ID11 pathogenicity** & whether it produces **toxins**
4) Inject **Biomarkers**[13] (i.e. **Neutrophil elastase**, NE) to NTHi infected CF tissues & **Florescent imaging** to observe **disease progression**, **lung function decline** and **bronchiectasis**.

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