Otitis Media
Are probiotics an answer ?

Dr Mary John[1]
Dr Eileen M Dunne[2]
Dr Paul V Licciardi[2,3]
Dr Catherine Satzke[2,4,5]
Dr Odilia Wijburg[5]
Prof Roy M Robins-Browne[4,5]
Prof Stephen O' Leary[1]

Department of Otolaryngology, The University *of Melbourne, Parkville, VIC, Australia[1], Pneumococcal Research[2], Allergy & Immune Disorders[3], Infectious Diseases & Microbiology[4], Murdoch Childrens Research Institute, Royal Children's Hospital, Parkville, VIC, Australia, Department of Microbiology & Immunology, The University of Melbourne, Parkville, VIC, Australia[5]*

Murdoch Childrens Research Institute
Healthier Kids. Healthier Future.

THE UNIVERSITY OF MELBOURNE

Outline

- Background
- Study Design
- *in vitro* Assays
- Future Directions

Murdoch Childrens Research Institute
Healthier Kids. Healthier Future.

THE UNIVERSITY OF MELBOURNE

Otitis Media (OM)

OM – one of the most common infection in children (Rovers et al 2004)

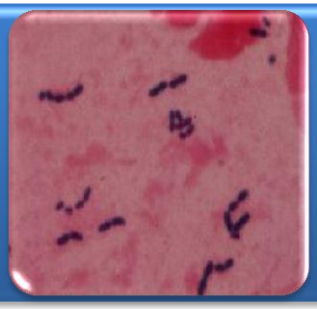
Aboriginal children – one of the highest prevalence of OM (Gunasekara et al 2008)

Starts as URI > acute OM > chronic OM (Kong et al 2009)

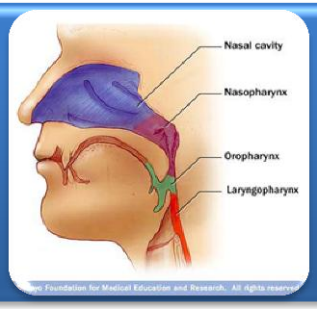
Murdoch Childrens Research Institute
Healthier Kids. Healthier Future.

THE UNIVERSITY OF MELBOURNE

Recurrent ear discharge > hearing loss > poor speech development (Williams et al 2009)



Streptococcus pneumoniae is one of the main bacteria causing OM (Stoodley et al 2008)



Colonise the nasopharynx > travel to middle ear > OM (Wiertsema et al 2009)

Murdoch Childrens Research Institute
Healthier Kids. Healthier Future.

THE UNIVERSITY OF MELBOURNE

How to tackle OM ?

- **Various strategies**

- ***S. pneumoniae***
- Early colonisation (3 m) - increased risk of OM (Faden et al 1997)
- Indigenous children earlier (3 wks) (Smith et al 2008)
- Increased bacterial load - increased risk of OM (Smith et al 2006)

- ***Can inhibition of colonisation be an alternative method in OM prevention?***

Probiotics

- Live micro organisms- confer health benefits to the host
- Possible mechanisms of action
  - Competitive inhibition of microbes
  - Immuno-modulation
  - Bacteriocin production (Iannitti et al 2010)
- Alpha haemolytic streptococci - inhibitory effect against OM pathogens
  (Tano et al 1999)

Murdoch Childrens Research Institute
Healthier Kids. Healthier Future.

THE UNIVERSITY OF MELBOURNE

Streptococcus salivarius

- Potential pharyngeal probiotic

  (Guglielmetti et al 2010)

- Safe, commercially available
- Produces bacteriocins, BLIS
- Harbours megaplasmids

  (Tagg et al 2009)

Murdoch Childrens Research Institute
Healthier Kids. Healthier Future.

THE UNIVERSITY OF MELBOURNE

Does *S. salivarius* inhibit the colonisation of *S. pneumoniae ?*

Murdoch Childrens Research Institute
Healthier Kids. Healthier Future.

THE UNIVERSITY OF MELBOURNE

Study Design

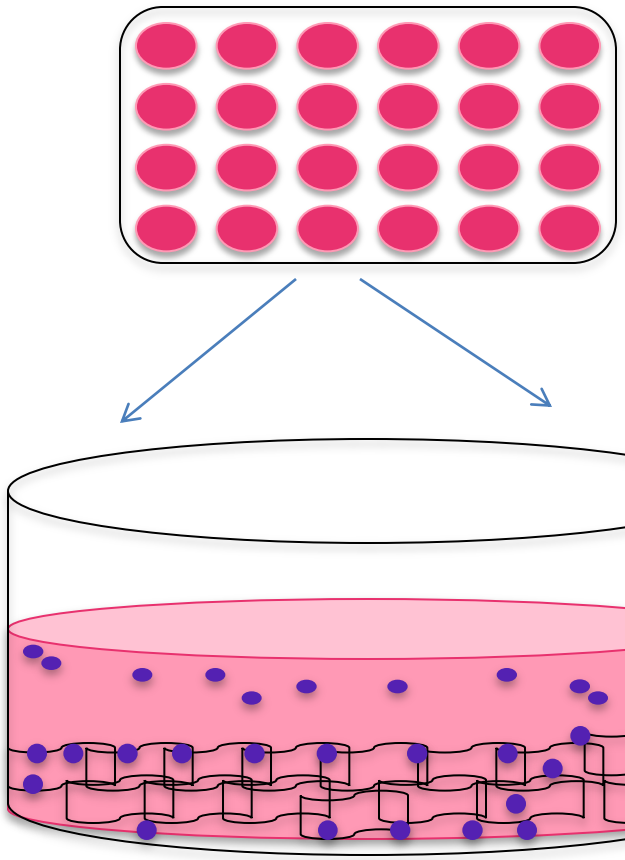
- *in vitro* lab assays
- *in vivo* animal experiments
- Clinical trial

in vitro Lab Assays

- Administration of ***S. salivarius*** will inhibit ***S. pneumoniae*** colonisation of **HEp-2 cells** in an in vitro laboratory setting

- Probiotic – ***S. salivarius*** **(Ss)**
- Bacteria – ***S. pneumoniae*** **(Pnc)**
- Epithelial cell- **HEp-2cell** (Human Epidermoid laryngeal carcinoma cell)

The Assay



24-well tray

Pneumococci
HEp-2 cells

Murdoch Childrens Research Institute
Healthier Kids. Healthier Future.

THE UNIVERSITY OF MELBOURNE

Various testing conditions

- cells & Pnc (standard)
- cells , Pnc & heparin (control)
- cells , Pnc & Ss (high)
- cells , Pnc & Ss (medium)
- cells , Pnc & Ss (low)

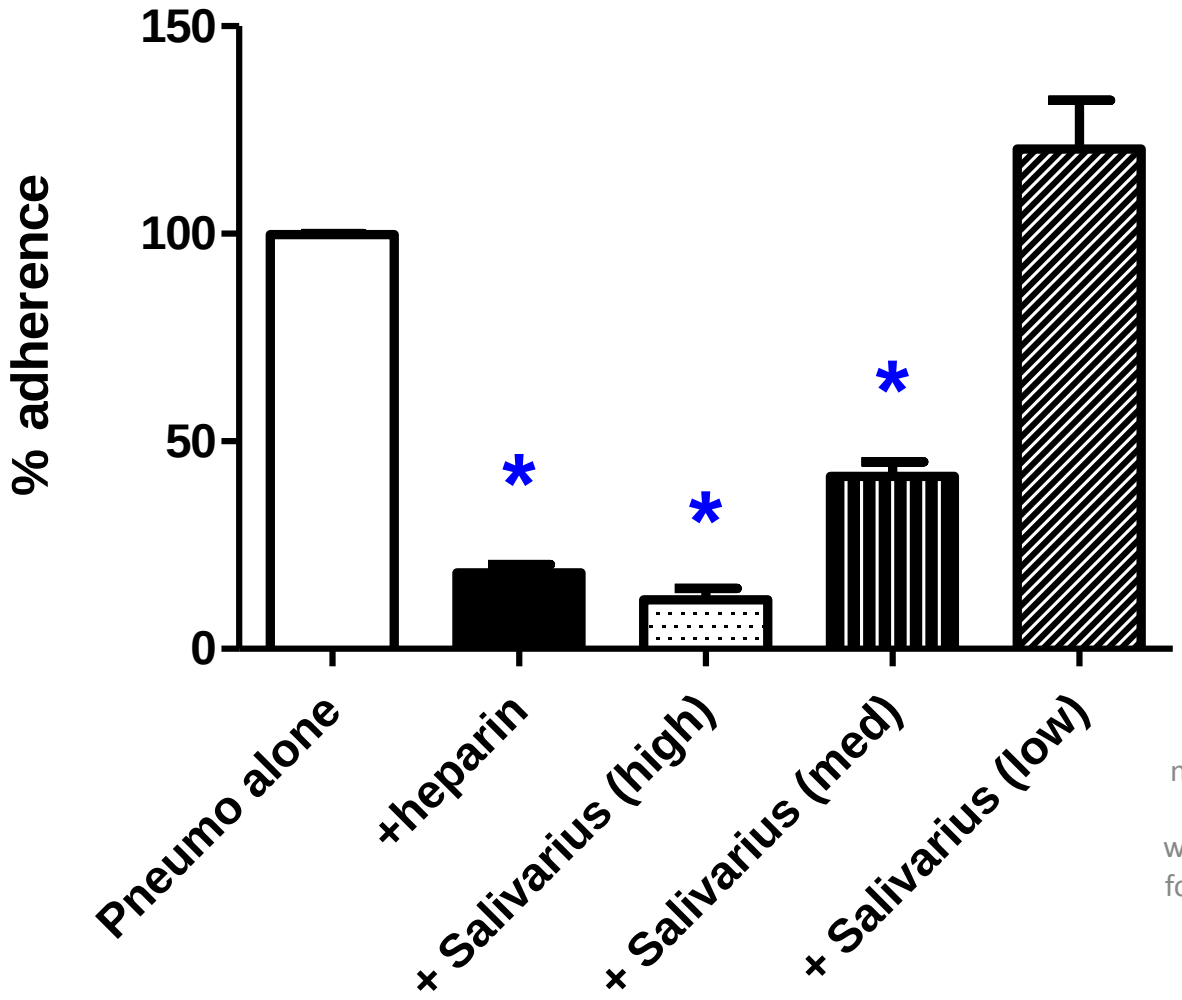
- **Quantification of Pnc** - qPCR

Addition of *S. salivarius*

- Pre-administration – one hour before Pnc
- Co-administration – along with Pnc
- Post-administration – one hour after Pnc

Results of *in vitro* assays

Pre-administration

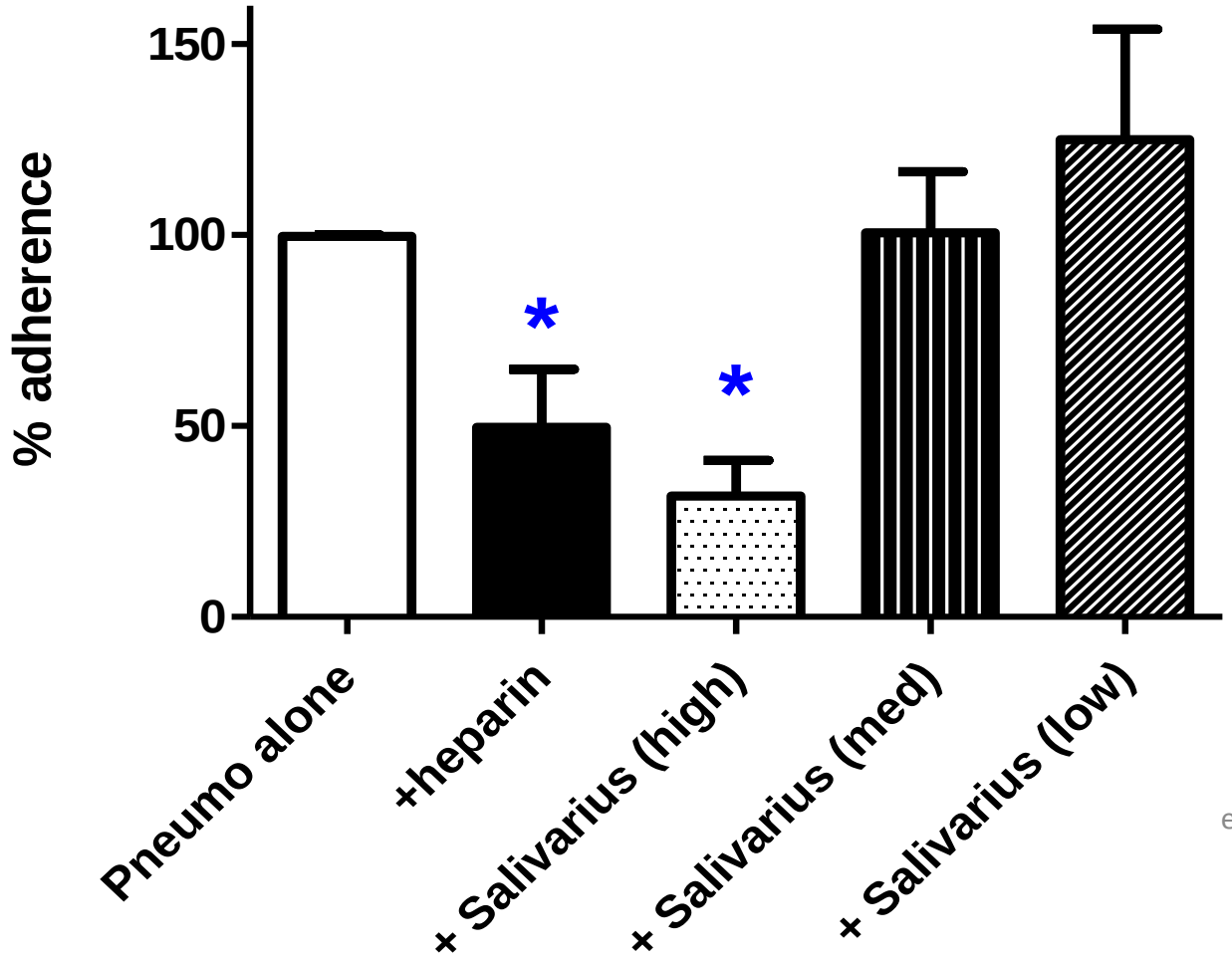


n=4; * indicates p<0.05 when compared to pneumo alone which was normalised to 100% for each assay (Students t test)

Murdoch Childrens Research Institute
Healthier Kids. Healthier Future.

THE UNIVERSITY OF MELBOURNE

Co-administration



n=3; * indicates p <0.05 when compared to pneumo alone which was normalised to 100% for each assay (Students t test)

Murdoch Childrens Research Institute
Healthier Kids. Healthier Future.

THE UNIVERSITY OF MELBOURNE

Conclusion

- Pre-administration and co-administration of *S. salivarius* show a dose dependent inhibition of *S. pneumonie* colonisation
- Pre-administration was more effective than co- administration
- Post administration – no effect

- On going experiments with 2 other serotypes

Future directions

- ***in vivo* mouse model experiment**
  - Does *S. salivarius* colonise the mouse?
  - Does *S. salivarius* inhibit the colonisation of *S. pneumoniae* ?
  - Does *S. salivarius* prevent OM in a mouse model?
- **Design a clinical trail**

Murdoch Childrens Research Institute
Healthier Kids. Healthier Future.

THE UNIVERSITY OF MELBOURNE

Thank you…

- My supervisors – Prof Stephen O'Leary,
  Prof Roy Robins Browne,
  Dr Catherine Satzke,
  Dr Odilia Wijburg,
  Dr Paul Licciardi

- Dr Eileen Dunne, Zheng Quan Toh
- Prof John Tagg and his colleagues
- Graeme Clark Foundation
- Hugh Noel Puckel Scholarship
- The University of Melbourne

Murdoch Childrens Research Institute
Healthier Kids. Healthier Future.

THE UNIVERSITY OF MELBOURNE

Clinical trial

- Does *S. salivarius* inhibit the colonisation of *S. pneumoniae* in the nasophaynx ?
- Does *S. salivarius* prevent OM?
- If *S. salivarius* is effective in OM, then

  - mode of administration
  - dose of administration
  - time of administration

Statistical Analysis

- Results were compared to pneumococcal adherence in wells containing *S. pneumoniae* alone, normalised to 100%. The student's t-test was used to evaluate differences % adherence between test conditions and S. pneumoniae alone. P values < 0.05 were considered statistically significant and are indicated with an asterisk.

qPCR

- Lyta gene specific for pneumococcus used

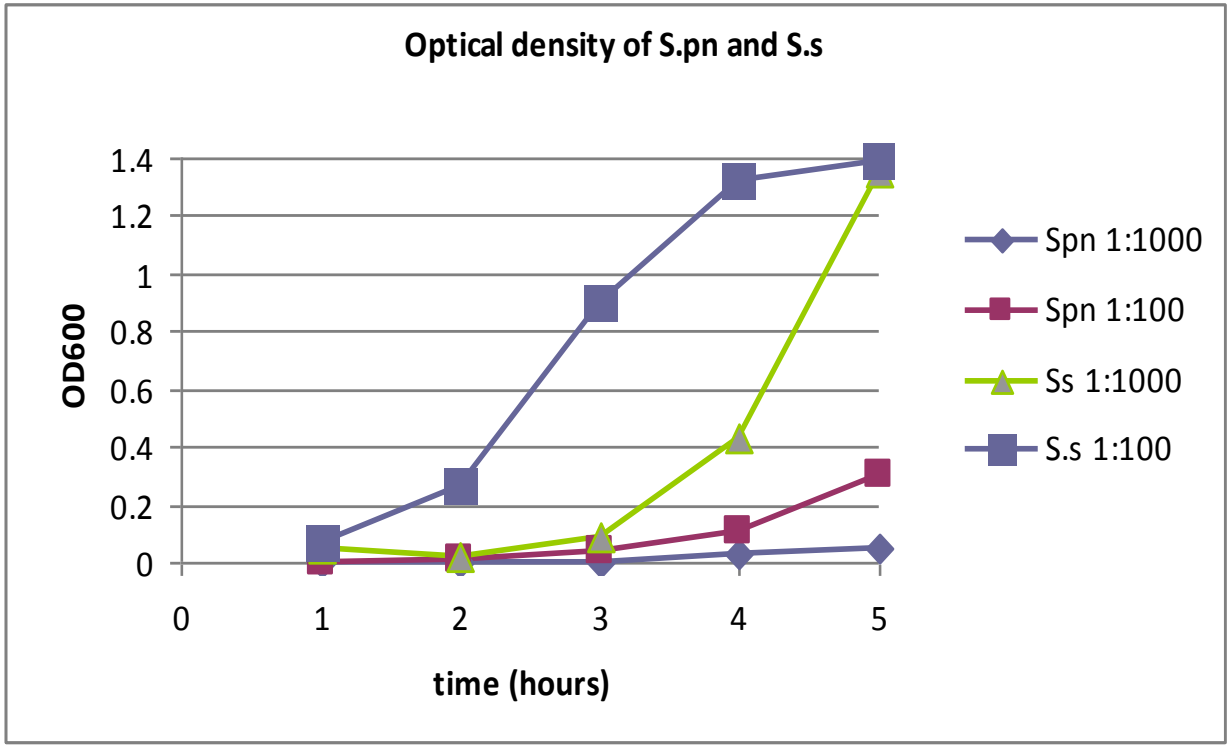


Figure 1. Growth of *S. pneumoniae* and *S. salivarius* over time as measured by optical density (OD_{600}).

Comparing qPCR to serial dilution and plating

	qPCR		Serial dilution & plating	
	CFU/ml PMP843	% colonisation	CFU/ml PMP 843	% colonisation
Pnc	**1.1×10^7**	**100**	**9.1×10^6**	**100**
Pnc + Heparin	**1.5×10^6**	**14**	**7.6×10^5**	**8**
Pnc + Ss (high)	**6.1×10^5**	**6**	**3.2×10^5**	**4**
Pnc + Ss (medium)	**3.6×10^6**	**33**	**2.7×10^6**	**29**
Pnc + Ss (low)	**9.8×10^6**	**92**	**8.2×10^6**	**90**

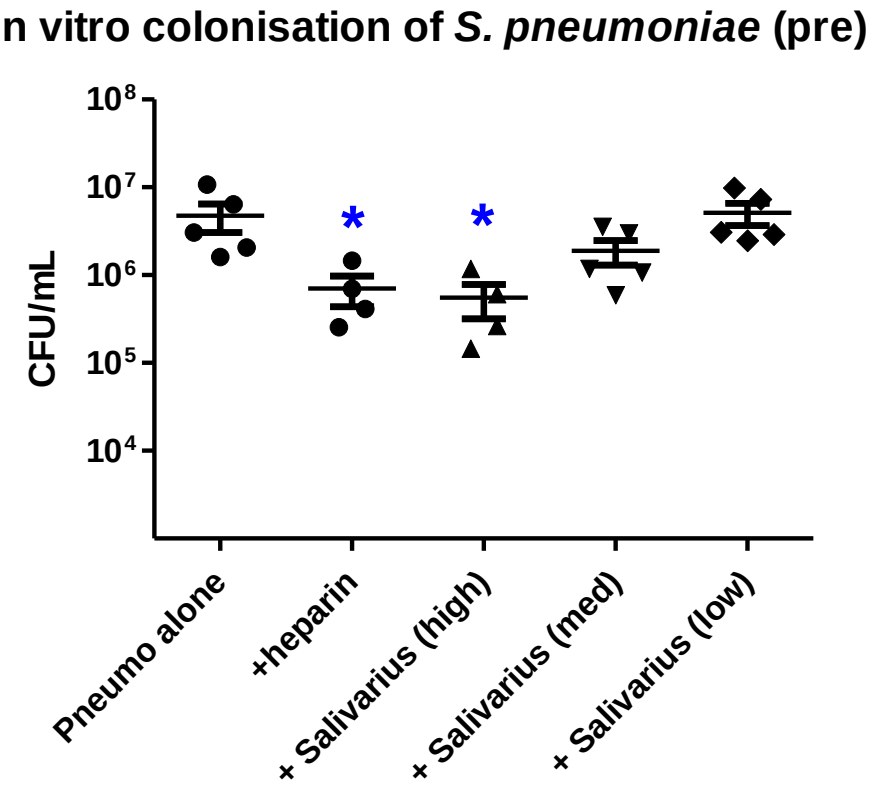


Figure 3. Colonisation of *S. pneumoniae* (CFU/mL) on Hep-2 cells when incubated alone, with heparin, or following pre-administration of high (5-10 *S. salivarius*: 1 *S. pneumoniae*), medium (1 *S. salivarius*: 1 *S. pneumoniae*), or low (1 *S. salivarius*: 5-10 *S. pneumoniae*) *S. salivarius*. * indicates $p < 0.05$ when compared to *S. pneumoniae* alone (Mann Whitney test).

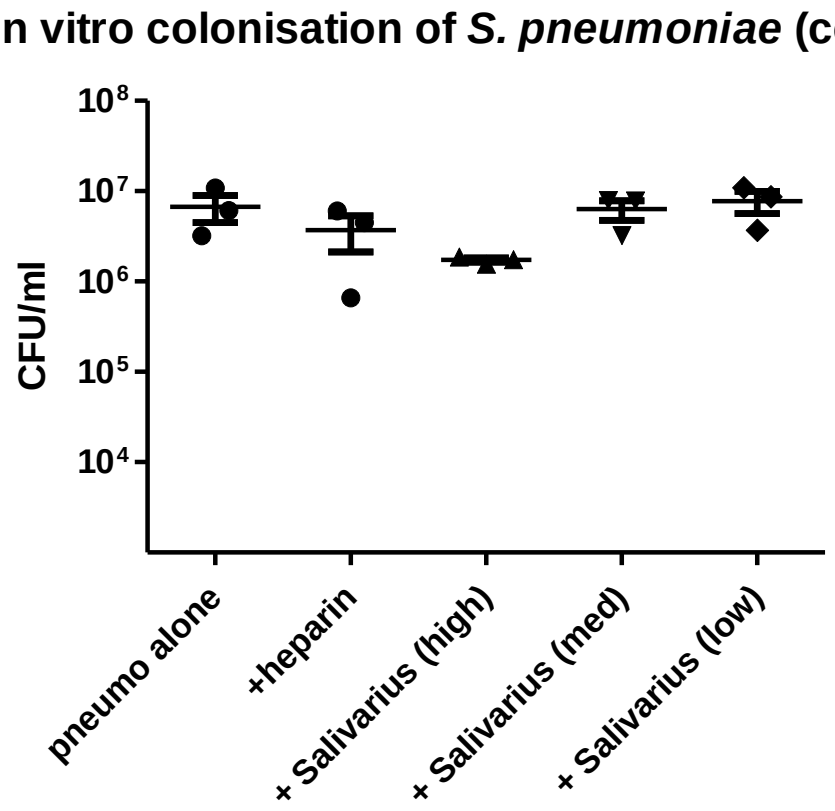


Figure 5. Colonisation of *S. pneumoniae* (CFU/mL) on Hep-2 cells when incubated alone, with heparin, or following co-administration of high (5-10 *S. salivarius*: 1 *S. pneumoniae*), medium (1 *S. salivarius*: 1 *S. pneumoniae*), or low (1 *S. salivarius*: 5-10 *S. pneumoniae*) *S. salivarius*.