Microbial interactions in an endotracheal biofilm

Surbhi Malhotra-Kumar, PhD
Laboratory of Medical Microbiology
Vaccine & Infectious Disease Institute
University of Antwerp, Belgium

Contents

- Why study the ETT biofilm?
- The ETT microbiome: molecular analysis and impact on development of ventilator-associated pneumonia (VAP)
- Mono- and -polymicrobial VAP
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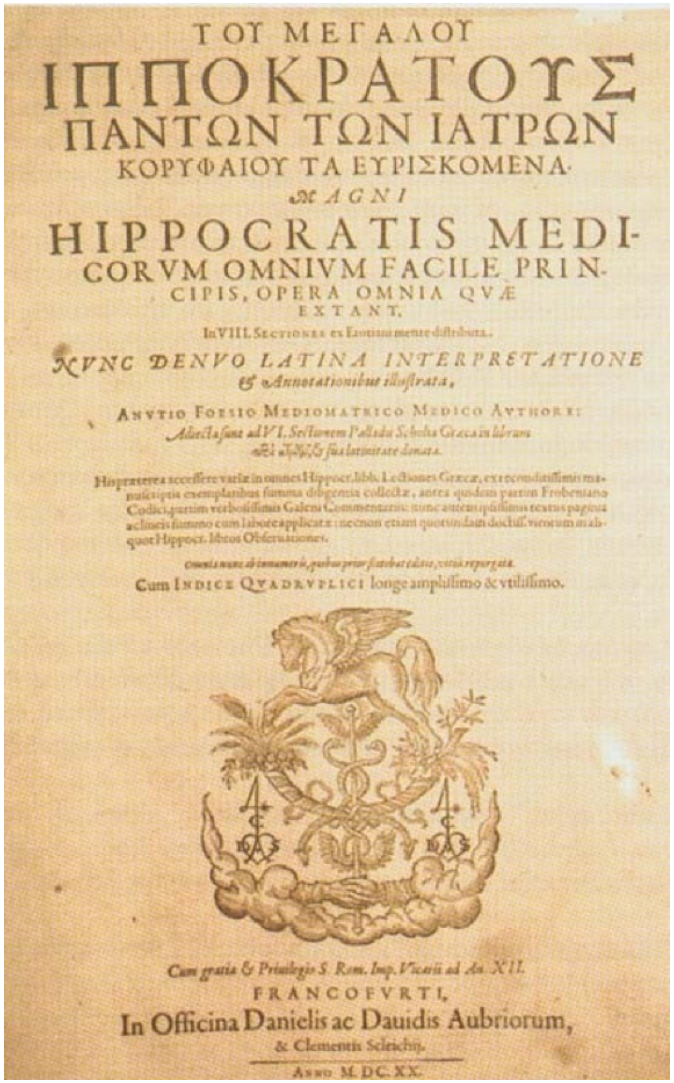
Why study the ETT biofilm?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MAGNI
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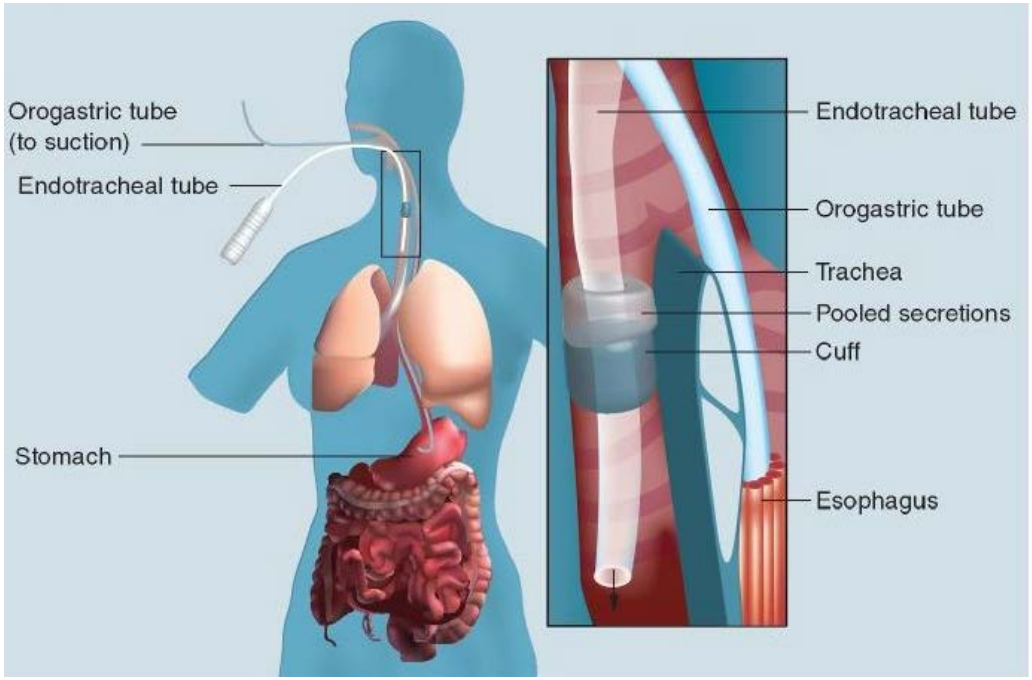
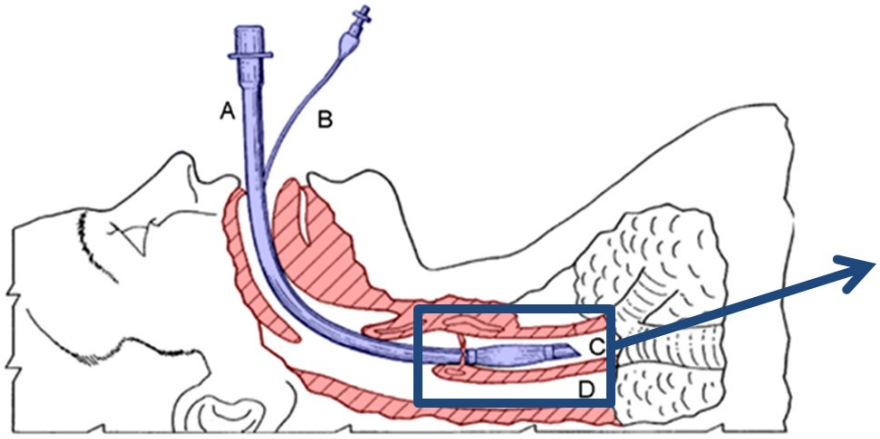
In VIII. Sectiones ex Erotiani mente distributa.
NVNC DENVO LATINA INTERPRETATIONE & Annotationibus illustrata,
ANVTIO FOESIO MEDIOMATRICO MEDICO AVTHORE:
Adiecta sunt ad VI. Sectionem Palladii Scholia Græca in librum
Cum INDICE QVADRVPLICI longe amplissimo & vtilissimo.
Cum gratia & Priuilegio S. Rom. Imp. Vicarii ad An. XII.
FRANCOFVRTI,
In Officina Danielis ac Dauidis Aubriorum,
& Clementis Schleichij.
ANNO M.DC.XX.

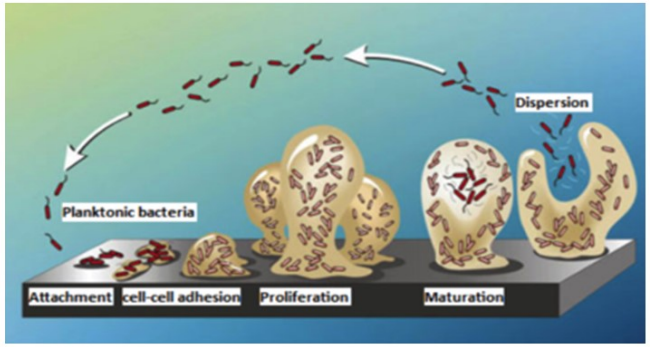


Biofilm formation on the endotracheal tube



Intubated patient: A: endotracheal tube; B: cuff inflation tube with pilot balloon; C: trachea; D: esophagus; E: cuff ; Patient material and micro-organisms can easily accumulate in the lower part of the ET tube around the cuff.

Biofilm formation



Bacteria discarded from the biofilm can make it to the lung and cause **ventilator associated pneumonia (VAP)**

P. aeruginosa
S. marcescens
Other bacteria

Bacterial colonization of the ETT

Anaesthesia. 1967 Apr;22(2):220-7.

Colonisation of the upper respiratory tract with Gram-negative bacilli after operation, endotracheal intubation and prophylactic antibiotic therapy.

Redman LR, Lockey E.

Crit Care Med. 1986 Apr;14(4):265-70.

Nosocomial pulmonary infection: possible etiologic significance of bacterial adhesion to endotracheal tubes.

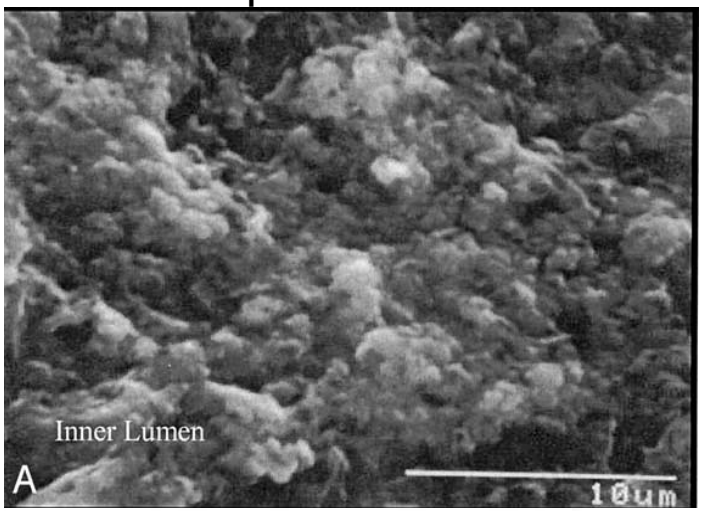
Sottile FD, Marrie TJ, Prough DS, Hobgood CD, Gower DJ, Webb LX, Costerton JW, Gristina AG.

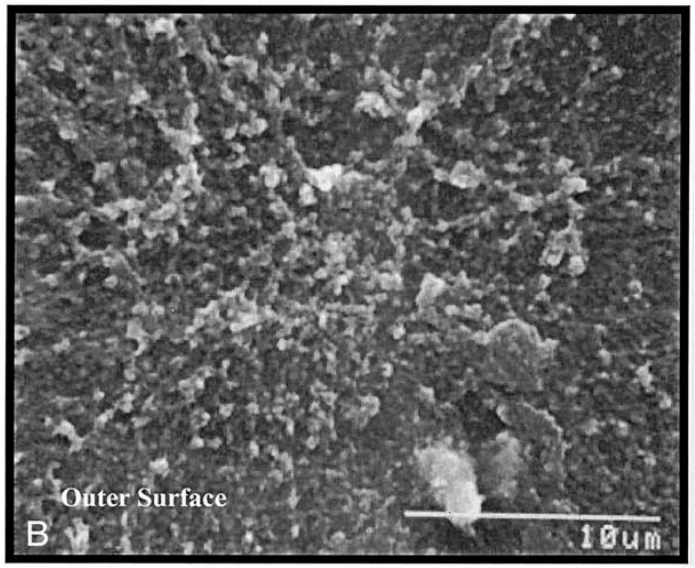
Abstract

Biomaterials are essential for life support and monitoring of critically ill patients, but their use increases the risk of nosocomial infection. Of the various plastics used for life support and monitoring devices, polyvinylchloride is one to which bacteria most readily adhere. Through the use of qualitative culture techniques and scanning and transmission electron microscopy, we studied the surfaces of polyvinylchloride **endotracheal** tubes removed from 25 ICU patients, to determine if bacterial adhesion to those tubes was sufficient to provide a possible source for repeated contamination of the tracheobronchial tree. Of the surfaces studied, 16% were partially covered and 84% were completely covered by an amorphous bacteria-containing matrix. Some **biofilm**-enclosed bacterial aggregates projected from the matrix into the lumen of the **tube**. The mechanism by which **endotracheal** tubes repeatedly inoculate the lungs of intubated patients may prove to be dislodgment of such aggregates by suction apparatus.

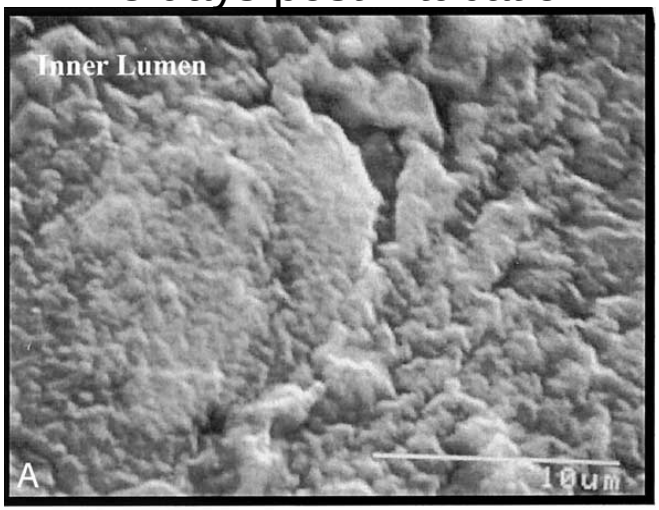
Is it really a biofilm?

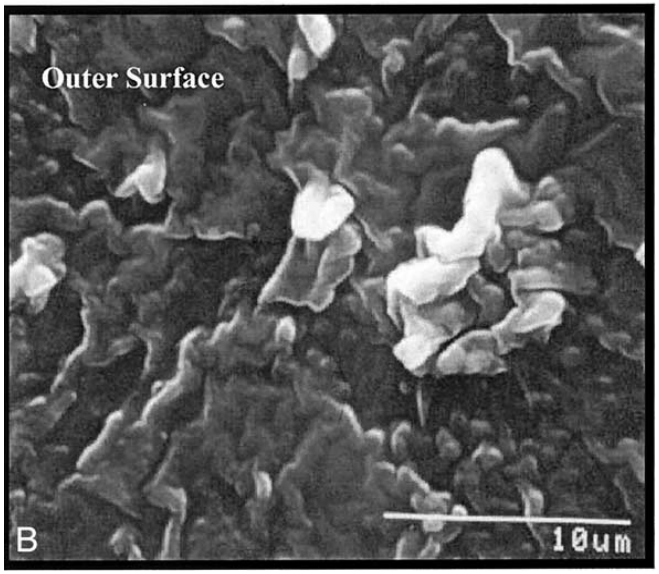
14 hrs post-intubation





3 days post-intubation





Sottile et al, 1986; Zur et al, 2004

Causative role of ETT biofilm in VAP

Table 2 Numbers of isolates of potential pathogens recovered from endotracheal tube biofilm and tracheal secretions

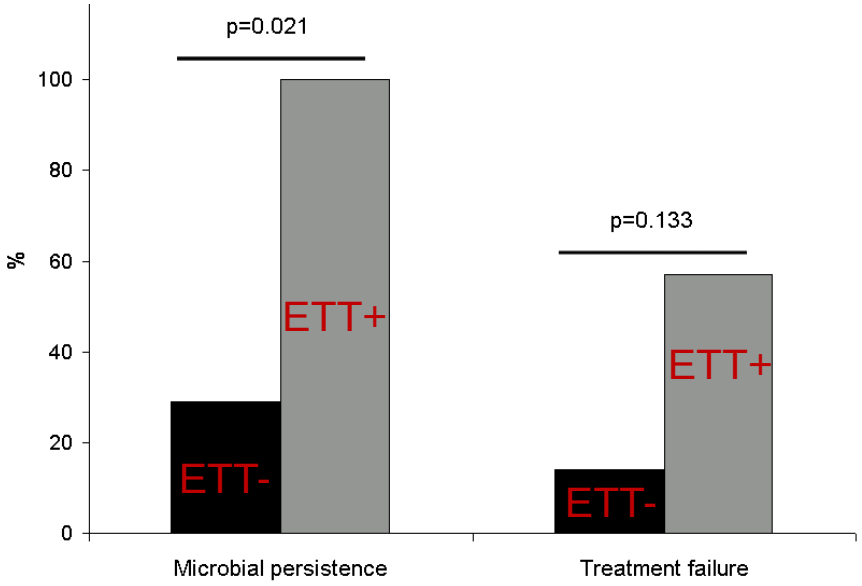
	Control		VAP	
	Biofilm	Tracheal	Biofilm	Tracheal
Pseudomonas aeruginosa	–	–	6	6
EGNB	2	3	4	5
Enterococcus faecalis	2	–	2	2
Staphylococcus aureus	2	–	6	6
Candida spp.	1	2	4	2

Adair et al, Int. Care Med., 1999

ETT biofilms are associated with microbial persistence and treatment failures

Table 2 Bacterial isolation in surveillance endotracheal aspirates

Microorganism	ETA n, %	Days until ETA+ (mean ± SEM)	ETA-ETT match (n, %)
Colonized patients	65, 87%	2.1 ± 0.4	36, 56%
Acinetobacter baumannii	20, 32%	7.8 ± 1.6	12, 60%
Pseudomonas aeruginosa	14, 22%	5.4 ± 2.1	9, 64%
Cocci (SCN, Streptococcus spp)	13, 20%	5 ± 0.9	4, 31%
Staphylococcus aureus (MSSA,MRSA)	10, 15%	2.2 ± 0.6	6, 60%
Candida albicans	29, 45%	2 ± 0.6	6, 21%
Candida no albicans	17, 26%	3.2 ± 0.5	1, 6%



Gil-Perotin et al, Crit. Care, 2012

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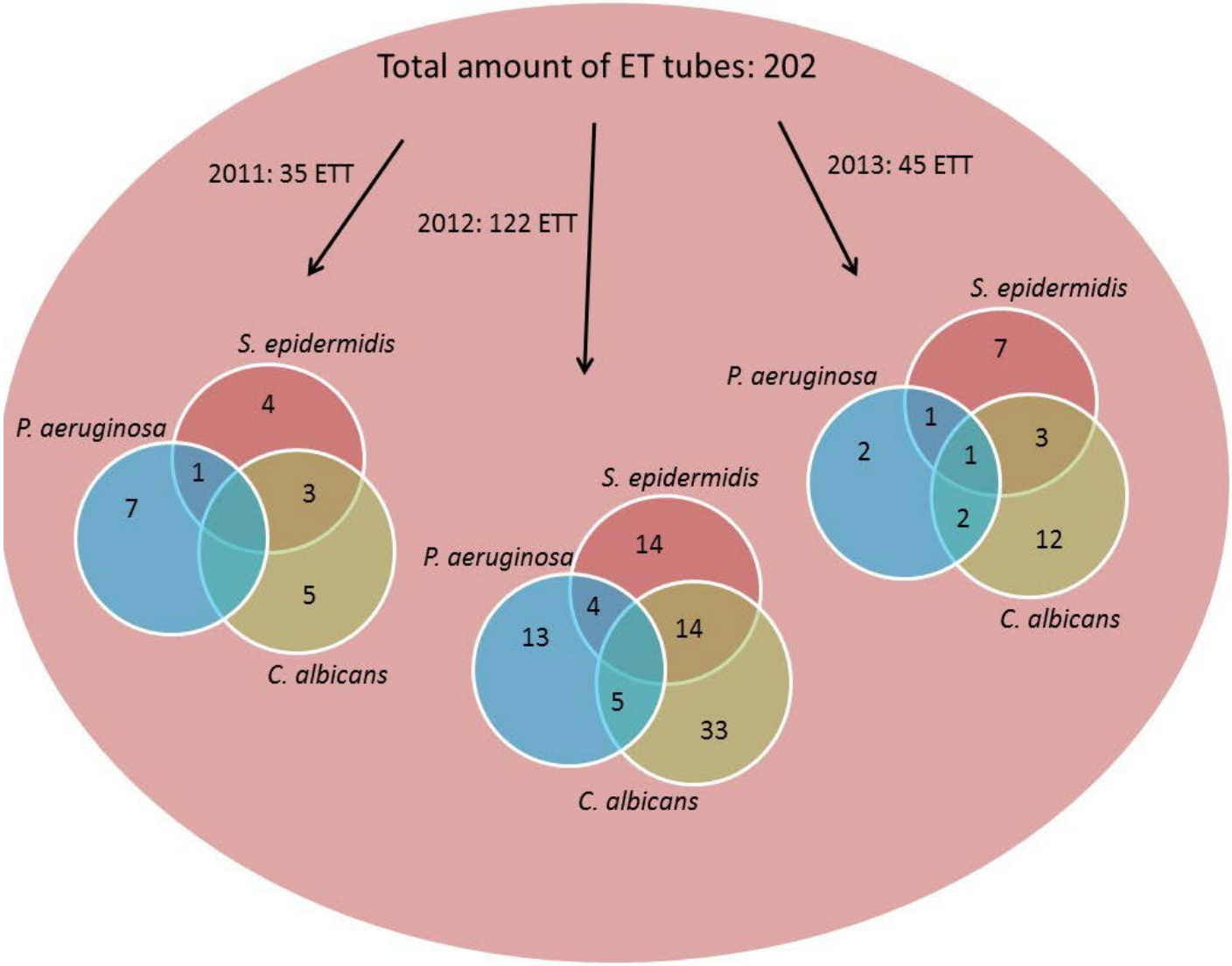
Molecular analysis of microbial communities in ETT biofilms

- 20 ETTs were analysed by 16S rDNA PCR-cloning-Sanger sequencing and 4 by pyrosequencing
- On average, 16S rDNA sequencing revealed 3 different species per ET biofilm
  - Simpson diversity indexes did not differ significantly between both methods (culture and sequencing of the clone libraries)
- Pyrosequencing analysis suggested that the four samples were dominated by members of the normal oral flora such as *Prevotella spp., Peptostreptococcus spp*. and lactic acid bacteria

Molecular analysis of microbial communities in ETT biofilms

- Inner luminal surface of 24 ETT from 20 patients analysed by quantitative culture and by denaturing gradient gel electrophoresis (DGGE) profiling of 16S rRNA gene
- DGGE profiling of the endotracheal biofilms revealed complex banding patterns containing between 3 and 22 (mean 6) bands per tube
- Significant inter-patient diversity
- No. of DGGE bands detected was not related to total viable microbial counts or the duration of intubation

Prevalence of potential pathogens in ET tube biofilms



Hotterbeekx et al, In preparation, 2015

Analysis of ETTs culture positive for *P. aeruginosa* or *S. epidermidis* or both

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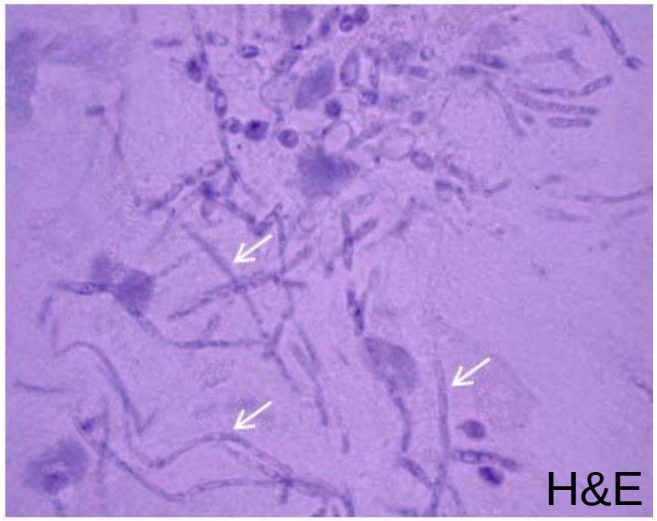
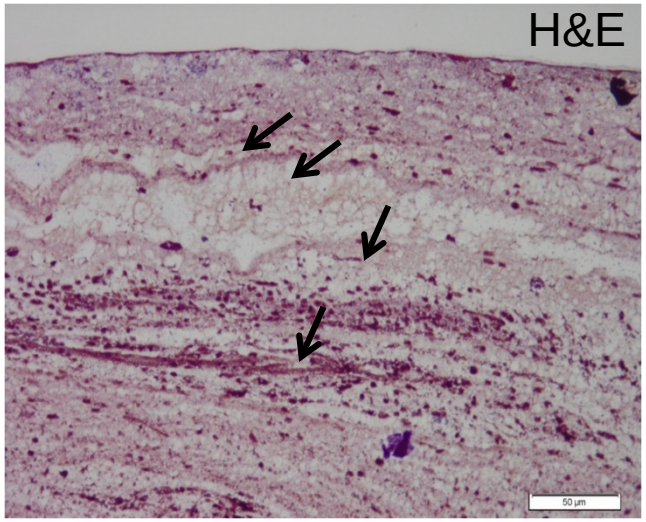
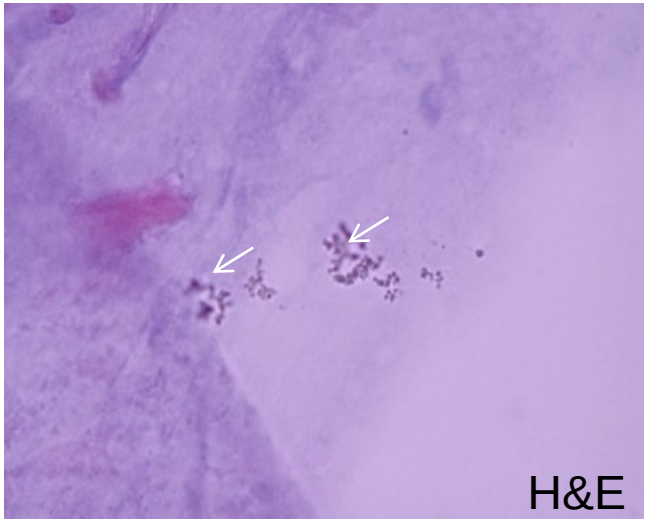
Histology of ETT biofilms

- Microtome sectioning
- Paraffin embedding
- Staining
  - H&E
  - Gram
  - DAPI

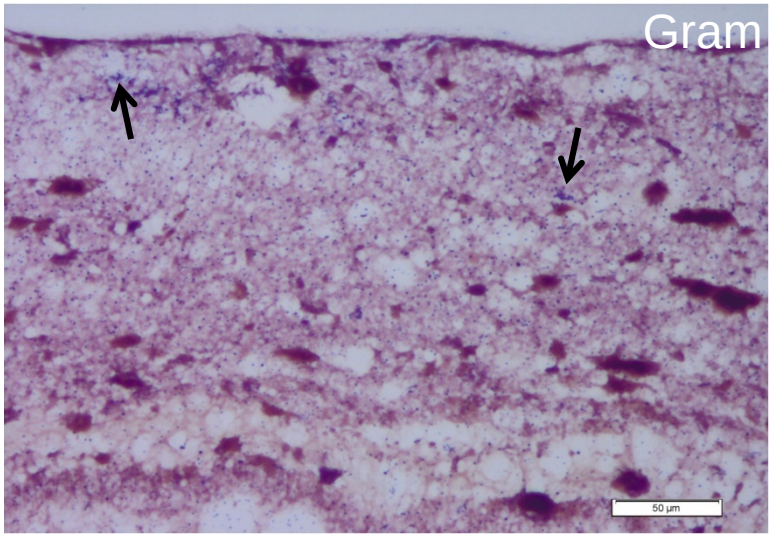


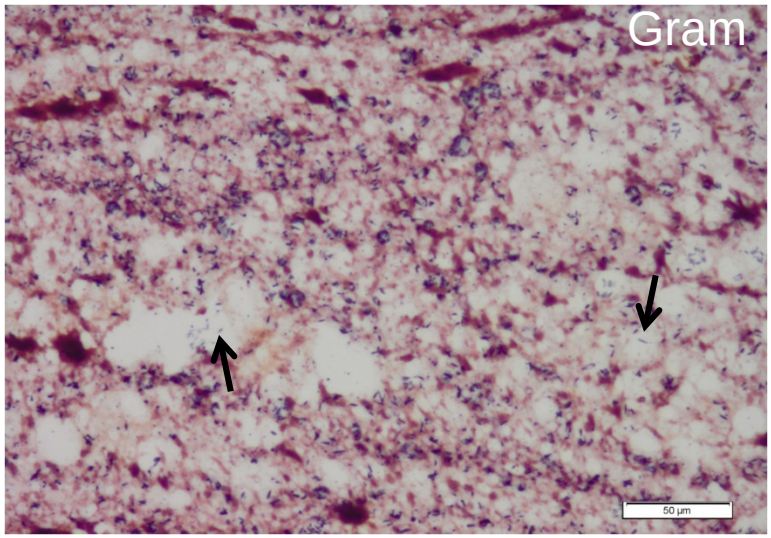


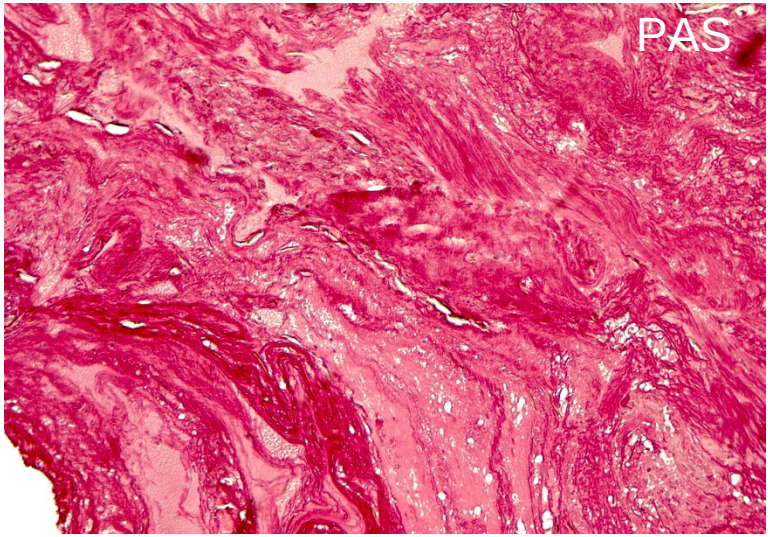


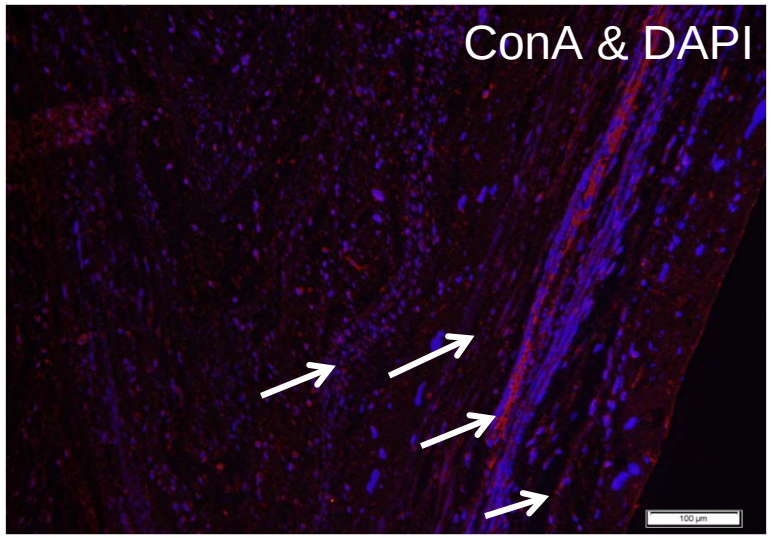
Courtesy S. Kumar-Singh, Vaxinfectio, Univ. Antwerp

Histology of ETT biofilms









Courtesy S. Kumar-Singh, Vaxinfectio, Univ. Antwerp

BAL-confirmed VAP and correlation with ETT culture

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Characterizing the ETT biofilm microbiome

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Core ETT microbiome

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Specific ETT microbiome

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Segata et al, Genome Biol., 2011; Hotterbeekx et al, In preparation, 2015

Organisms associated with *S epidermidis* in ETT biofilms

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Development of VAP and patient survival upon extubation: a cluster analysis

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Hotterbeekx et al, In preparation, 2015

Impact of the ETT microbiome on patient survival

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Segata et al, Genome Biol., 2011; Hotterbeekx et al, In preparation, 2015

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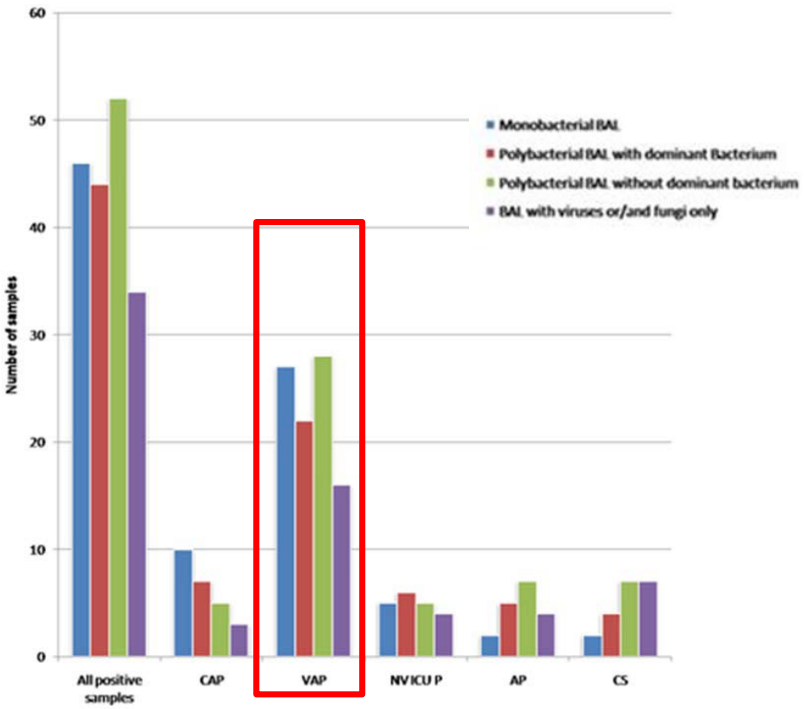
Is ICU pneumonia (VAP) polymicrobial?

- 3 year study in HAP and CAP patients and controls
- 25% BAL samples monomicrobial and 75% polymicrobial from pneumonia patients

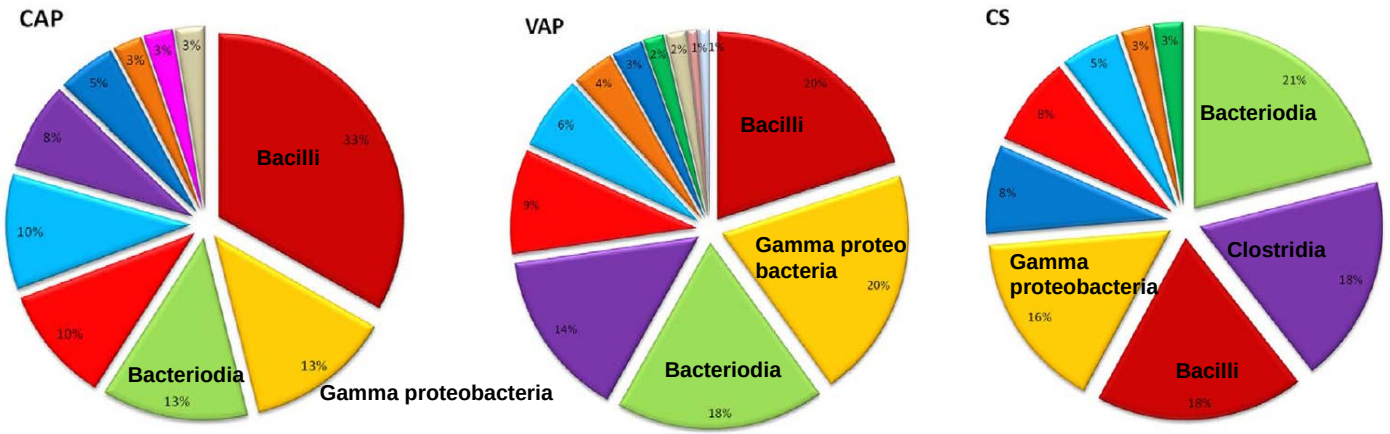


	Pneumonia cohorts	
	CAP (n = 32)	VAP (n = 106)
Only bacteria	12	34
Only fungi	0	2
Only viruses	3	9
Bacteria and fungi	3	4
Bacteria and viruses	6	33
Fungi and viruses	0	4
Bacteria and fungi and viruses	1	7
Total positive samples	25	93

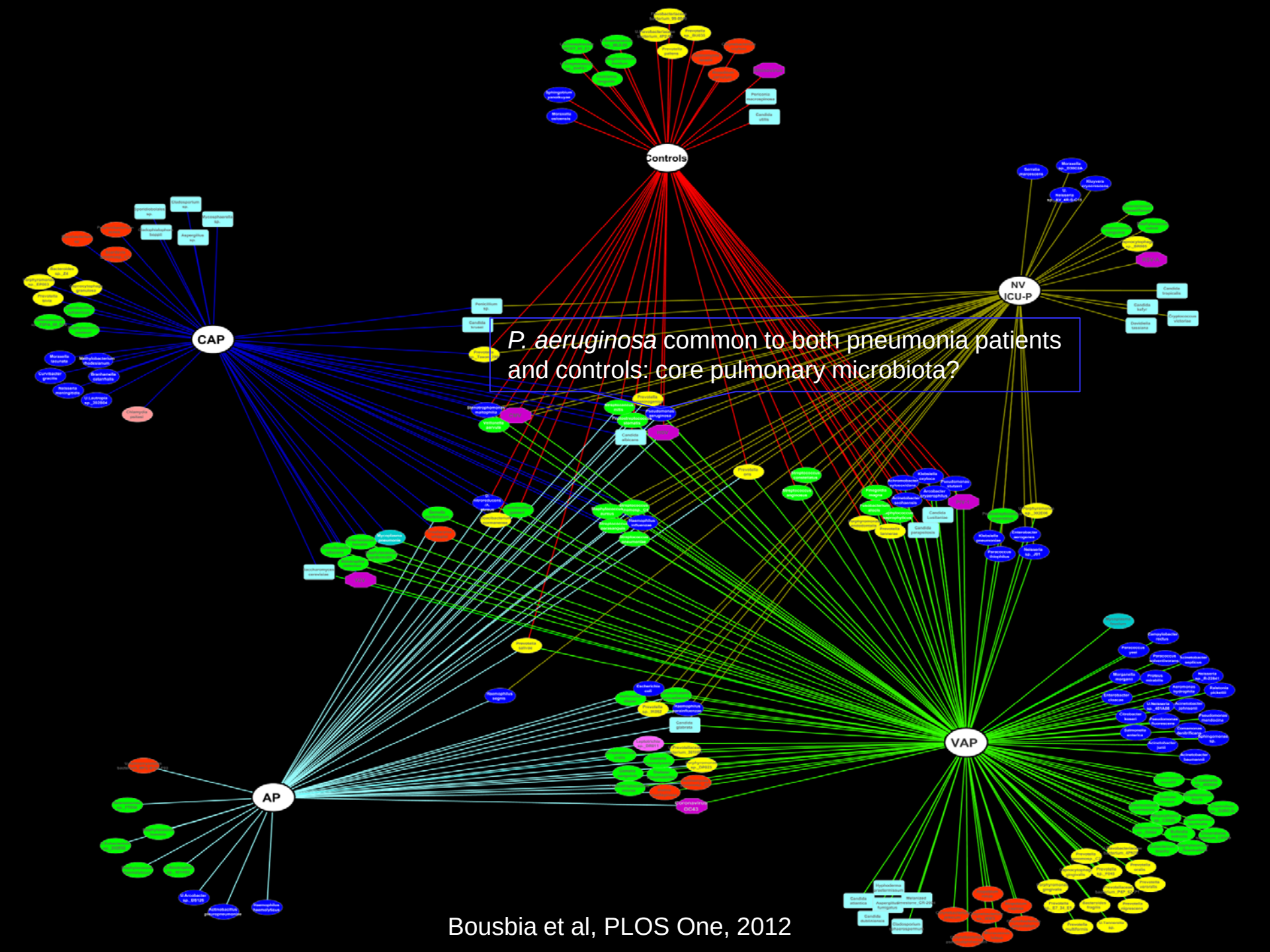
Bousbia et al, PLOS One, 2012

Characteristics of mono- and -polymicrobial VAP

	Pneumonia patients (n = 135/185)		*Control subjects (n = 22/25)*	
	Monomicrobial	**Polymicrobial**	**Monomicrobial**	**Polymicrobial**
Case number	32	82	3	15
Temperature, °C (SD)	37.6 (1)	37.8 (1.7)	37.2 (1.2)	37.6 (1.4)
Initial antibiotic therapy	18	33	2	9
Less than 2 days prior to sampling	6	17	0	3
3 days or more prior to sampling	12	16	2	6
Length of ICU stay prior to sampling, d (SD)	6.1 (9.1)	6.3 (10)	1.6 (1.1)	13.1 (15.6)
Total length of hospital stay, d (SD)	26 (29.7)	28.2 (23.3	4.3 (1.5)	36.6 (35.9)
Length of MV prior to sampling, d (SD)	6.8 (10.3)	7.1 (11.2)	1.3 (0.5)	6.3 (7.2)
Sepsis	7	19	0	3
Septic shock	15	38	1	7
ICU mortality (%)	16 (50)	23 (28)	1 (33)	3 (20)

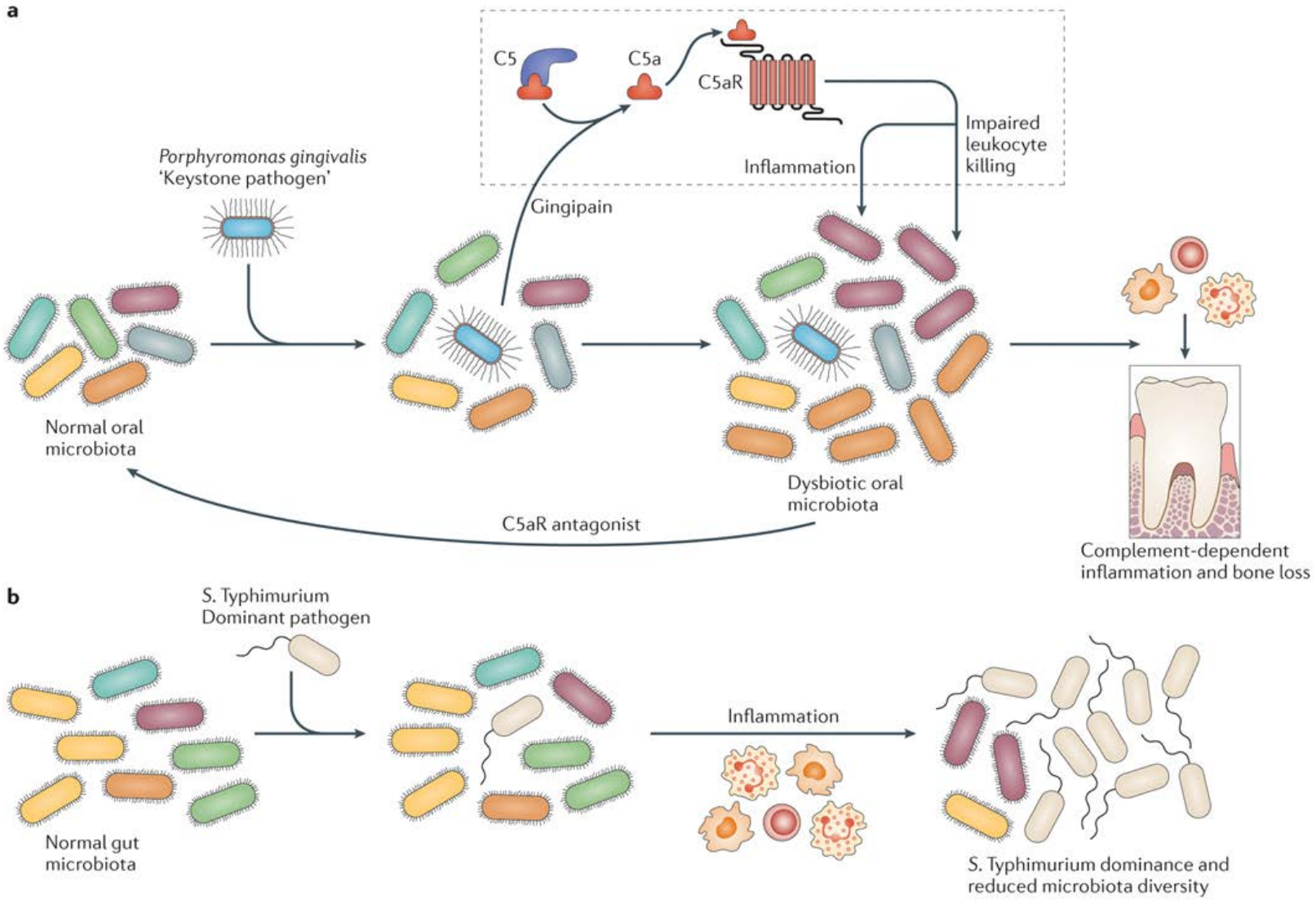


Bousbia et al, PLOS One, 2012



Bousbia et al, PLOS One, 2012

Microbial consortium interactions: ‘Keystone' and dominant pathogens



Hajishengallis et al, Nat. Rev. Microb., 2012

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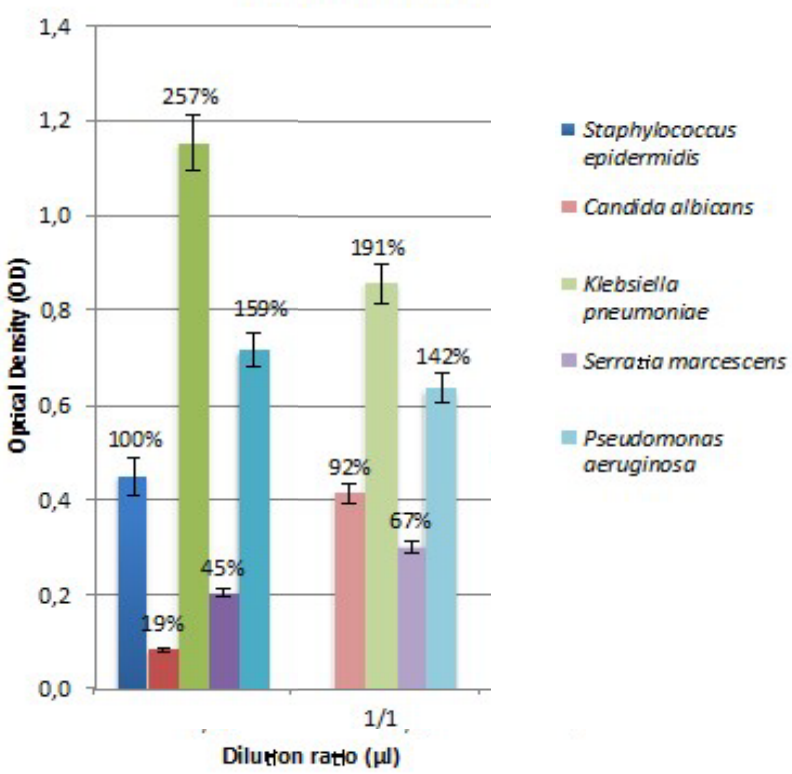
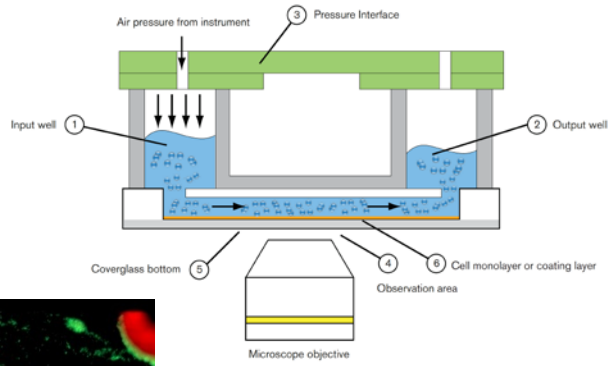
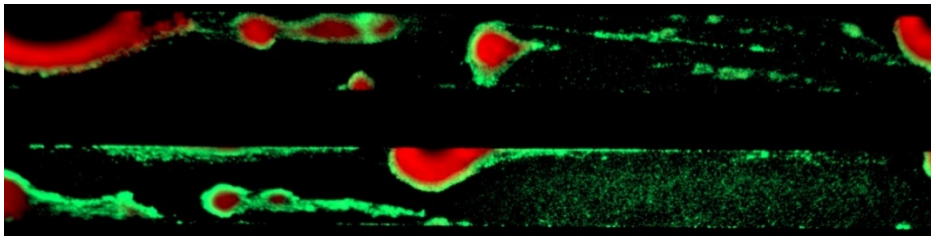
In vitro modelling of ETT microbial interactions



Static assay

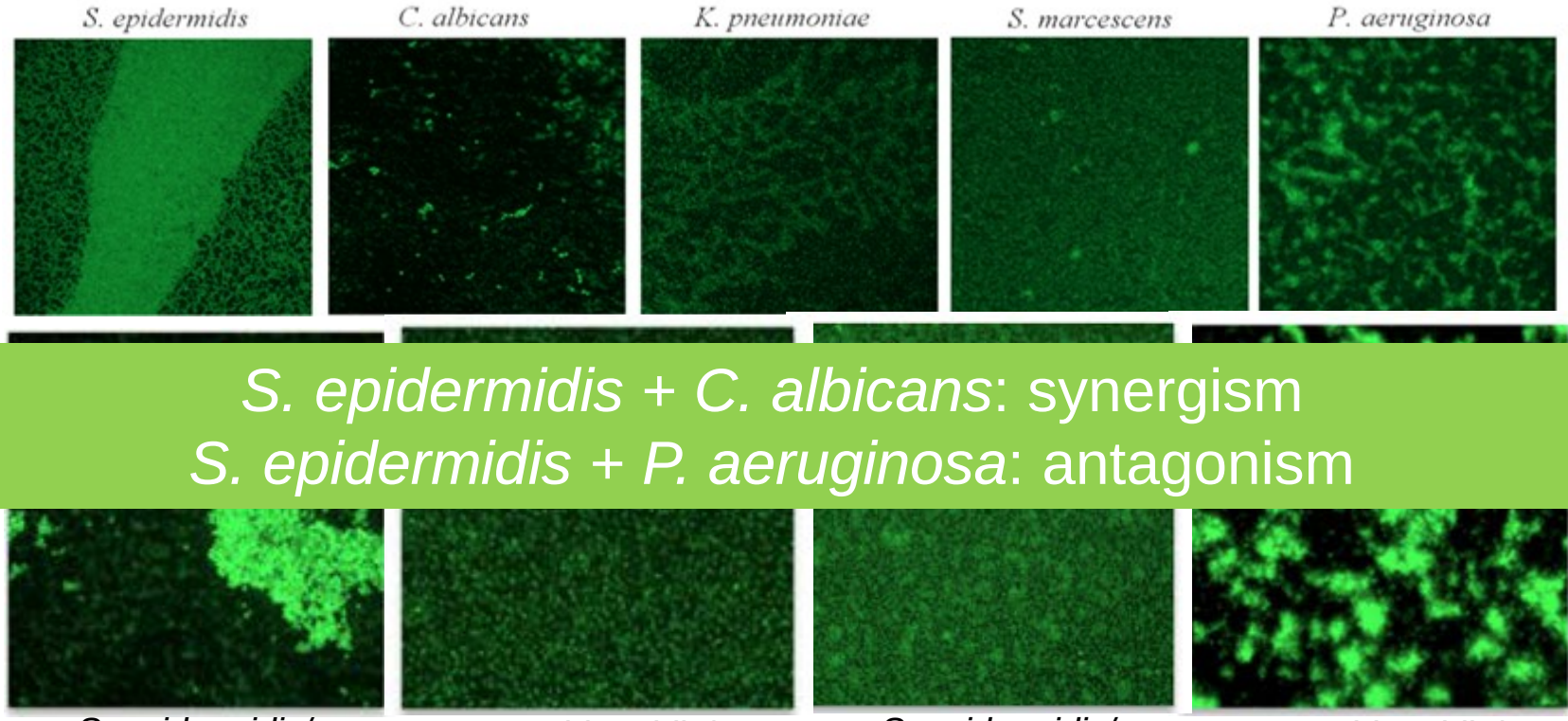




Bioflux 200, Fluxion

De Backer et al, 2015

In vitro modelling of ETT microbial interactions



S. epidermidis + *C. albicans*: synergism

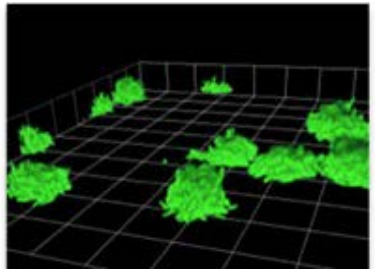
S. epidermidis + *P. aeruginosa*: antagonism

S. epidermidis/ C. albicans

S. epidermidis/ S. marcescens

S. epidermidis/ K. pneumoniae

S. epidermidis/ P. aeruginosa



De Backer et al, 2015

Effect of biofilm supernatant of *P. aeruginosa* and *S. marcescens* on *S. epidermidis* biofilms

Hotterbeekx et al, In preparation, 2015

In vivo modelling of ETT microbial interactions

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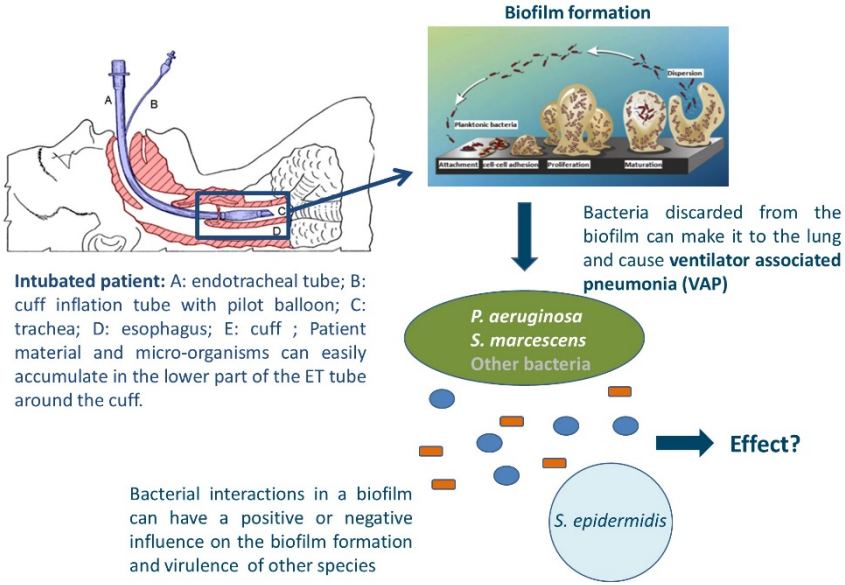
Courtesy S. Kumar-Singh, Vaxinfectio, Univ. Antwerp

Some conclusions



- Need for more comprehensive ‘Big data’
  - Role of accessory organisms in VAP etiology
- ETT fingerprint as a prognostic and, possibly, diagnostic marker
- Rapid molecular diagnostic tools to include fastidious ‘keystone’ and ‘dominant’ pathogens

Laboratory of Medical Microbiology,
University of Antwerp

An Hotterbeekx 

Basil Britto Xavier 

Christine Lammens 

Pieter Moons 

Sarah De Backer 

University Hospital &
University of Antwerp/Vaxinfectio

Prof. P. Jorens
Prof. G. Ieven
Prof. S. Kumar-Singh
Prof. H. Goossens

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fwo Opening new horizons

iwt agency for Innovation by Science and Technology