

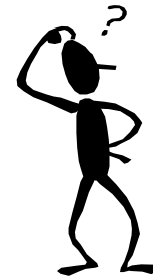
# Biofilm in bacteria



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- Who can **form biofilm** (bacteria, viruses, yeasts, 1species, more species, multibacterial community)
- Where **is the biofilm** (almost on solid surfaces)
- How do microbes **form biofilm** (difficult mechanism)
- Why do they **form biofilm** (guard against various circumstances)



# Who can form a biofilm?

- *P. aeruginosa*
- *S. coagulase negative*
- *K. pneumoniae*
- *E. coli*
- *S. aureus*
- *E. faecalis*
- *A. baumannii*
- *almost all bacteria, yeasts and fungi*



# Where do we find biofilm?

- ☞ Catheters (bloodstream..)
- ☞ Contact lens
- ☞ Teeth implantates
- ☞ Medical devices
- ☞ Water tubes
- ☞ Plaque on teeth, higher consumption of sugars + minimum teeth brushing are the cofactors in dental caries formation
- ☞ Stones in river – physiological!

# Stages of biofilm development

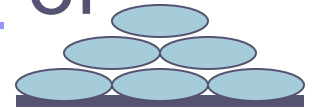
- Direct contact of a planktonic bacteria with a surface



- Attachment to this surface



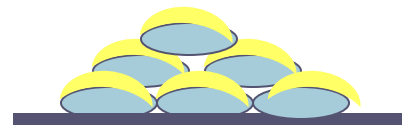
- Adhesion, growth, and aggregation of cells into microcolonies



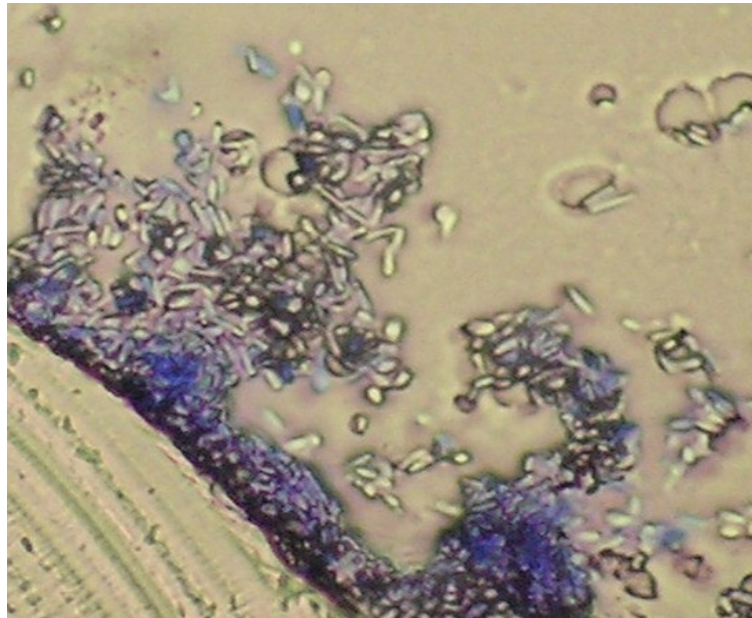
- Production of polymeric matrix



- Formation of three-dimensional structure known as a biofilm



# Biofilm formation in time



available on [www.medmicro.info](http://www.medmicro.info)

A black stick figure is shown in a thinking pose, with its right hand on its chin and a question mark above its head.

# Main importance of biofilm formation

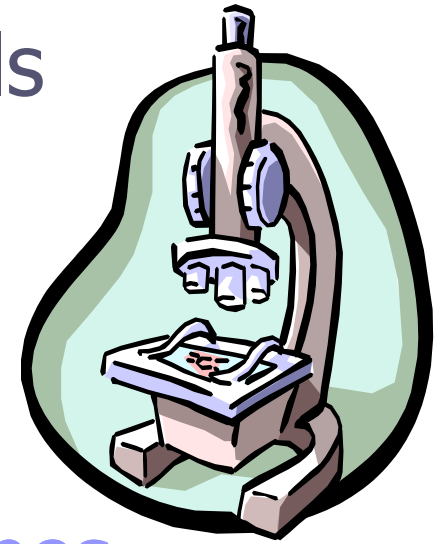
Bacteria harbored inside are protected against:

- ❖ antibiotic action
- ❖ host's immune response
- ❖ disinfection



# The inefficiency of antibiotics may be due to:

- ☛ Polyanionic charge of sessile cells
- ☛ Decreased bacterial growth
- ☛ Diffusion barrier of glycocalyx
- ☛ Reaction with biofilm matrix
- ☛ Formation of protected phenotypes
- ☛ Mechanism of intercellular signalling
- ☛ Host's immune response mechanisms...





# Biofilm detection

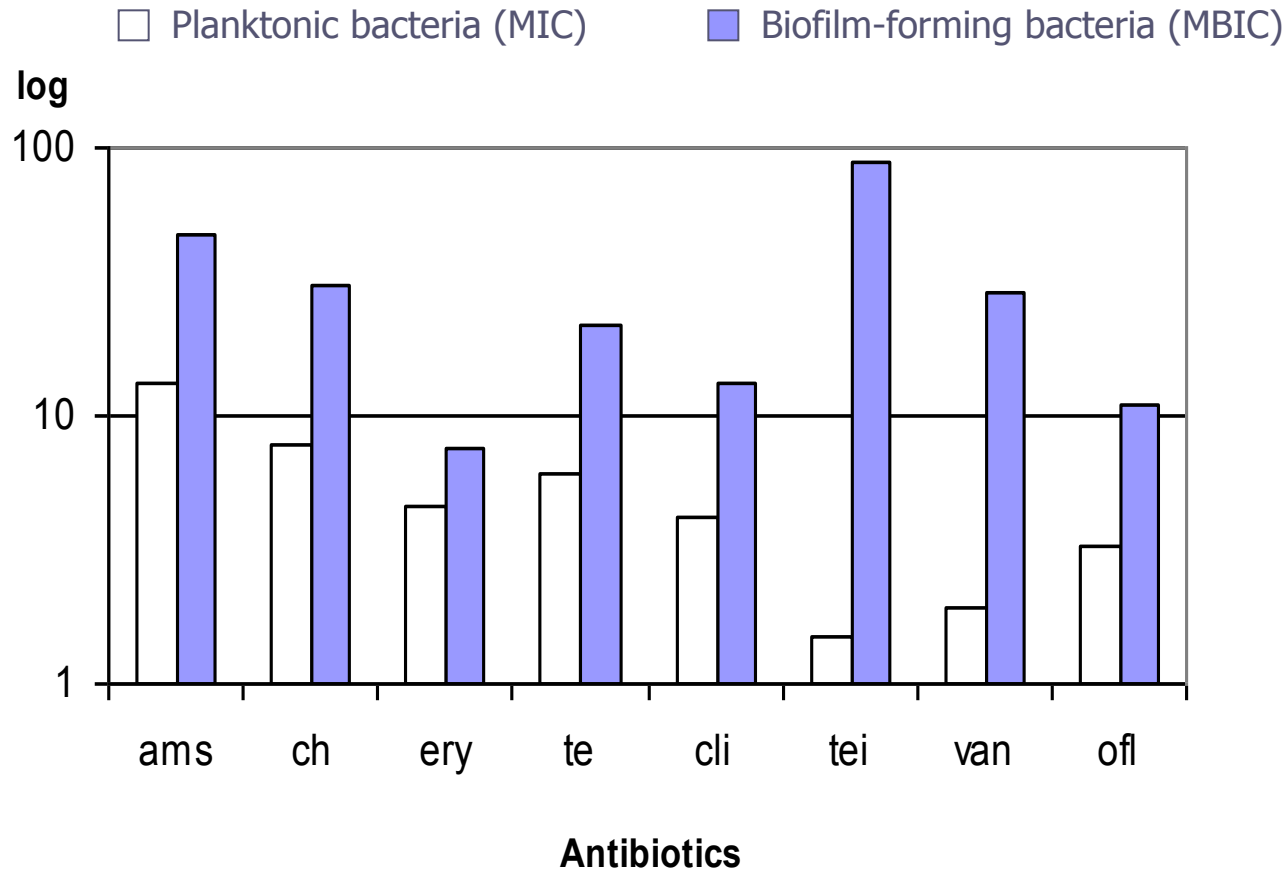


- ❖ **In test tubes or in plate via Christensen<sup>1</sup>** cultivation, washing of planktonic bacteria + staining of adhering bacteria – blue colour on whole tube wall not only a ring or sediment – it is a biofilm!
- ❖ **Detection in tubes stained with alcian blue**
- ❖ **El. microscopy, SEM, TEM**
- ❖ Special **cultivators** and phenotypic methods
- ❖ **Genotypic methods (PCR, FISH) etc.**

# Biofilm susceptibility testing

- ❖ Cultivation of wild-type **bacteria** v “microtitate” **plate in broth**
- ❖ Washing of planktonic bacteria + adding of diluted antibiotic (**combination of ATB**) and cultivation
- ❖ **MBEC** (minumum biofilm eradication concentration) was determined
- ❖ **MBEC** was compared with **MIC** (minimum inhibitory concentration)
- ❖ **Synergy testing of 2 antibiotics: FBIC** (fractionate biofilm inhibitory concentration) was determined in special laboratories
- ❖ Clinical relations

# MIC vs. MBEC values



Abbreviations: ampicillin/sulbactam, ch - chloramphenicol, ery - erythromycin, te - tetracycline, cli - clindamycin, tei - teicoplanin, van - vancomycin, ofl - ofloxacin

# Antibiotic susceptibility of *S. aureus* no. 351

351	MIC	MBEC
amp/sulbact.	0,125*	2*
tetracyclin	2*	64
clindamycin	2*	32
ofloxacin	0.25*	>8
teicoplanin	0.031*	>8
vancomycin	0.5*	4*

<i>S. aureus</i> 351	$\sum \text{FBIC} \leq 0.5$ strong SE	$\sum \text{FBIC} > 0.5a \leq 1$ partially SE	$\sum \text{FBIC} > 1a \leq 4$ indifferent	$\sum \text{FBIC} > 4$ antagonism
Antibiotic combinations				
clindamycin+vancomycin			2,00	
clindamycin+tetracyclin		1,00		
ofloxacin+teicoplanin			1,30	
teicoplanin+ampicillin/sulbactam	0,38			
vancomycin+tetracyclin			2,00	
ampicillin/sulbactam+ofloxacin			2,00	

\* susceptible according to conventional MIC evaluation. MBIC 4x - 256x higher than MIC (mg/l)



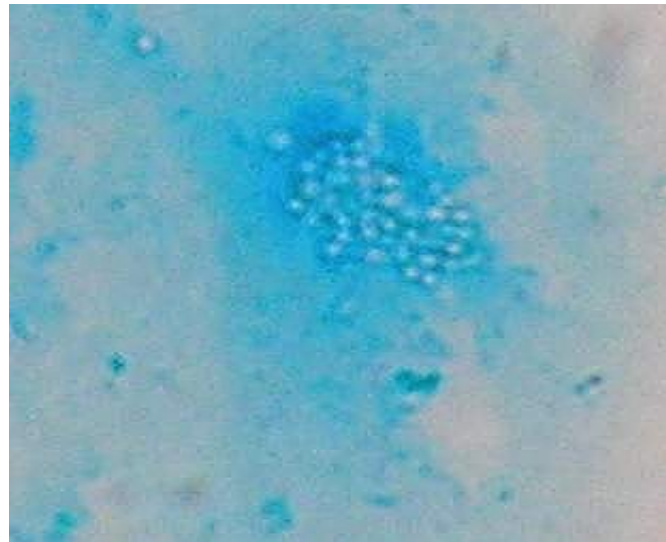
# Results + eradication

- ☞ **MBIC** values are often **higher** than **break point** for tested antibiotics (bacteria are resistant) + also **higher** than MIC
- ☞ 1 ATB does not work? Use **ATB combination** - **synergy testing** can be also used for **multiresistant** planktonic bacteria (with modifications)
- ☞ Resistance to **ATB combinations?** **Explantation of a biofilm focus** (removing of catheters, joint/teeth implantates)
- ☞ **Prevention:** catheters **coated with antimicrobial substances** - minocyclin and rifampicin, washing, hygiene...



# 1 – Microscopy of oral biofilm

- ☞ Gramm staining - G +, G- bacteria, host cells (epitelium, leukocyte etc.)
- ☞ Alcian blue stains polysaccharide material



# 2 – Teeth brushing and oral biofilm

Volunteer gum tablette with colour staining the teeth plaque. Tablette stay in oral cavity for 2 min. Draw the plaque





# 3- Catheters

- **Classic cultivation in broth:** We detect only bacteria in planktonic form. *Bacteria in biofilm won't leave the catheter wall*
- **Semiquantitative method:** We detect bacteria on surface of a catheter and can semiquantitative count them, *but we can not detect bacteria inside the lumen*
- **Sonification:** destroys biofilm on catheter surface as well as on catheter inside. Inoculation of specific sample volume *enable to determinate quantity of* microbes

# 4 – Presence of saccharides in food and teeth plaque formation

- Look at various amount of saccharides in food and biofilm formation speed in cariogenennous *Streptococcus mutans*.  
*What ´s happen?*

*Higher amounts of saccharides in food + higher length of staying in oral cavity- higher plaque.....!*

# 5 – MIC versus MBEC

- MIC - minimum inhibitory concentration of ATB (planktonic form)
- MBEC - the lowest concentration of antibiotic, where is the eradication of a biofilm possible (any living cells, pH without change - row stay red) (for biofilm forming bacteria).