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Gram-positive versus Gram-negative biofilms – common grounds and essential differences

Oana Săndulescu, MD, PhD

Associate Professor, Infectious Diseases

Carol Davila University of Medicine and Pharmacy, Bucharest, Romania

National Institute for Infectious Diseases "Prof. Dr. Matei Balș", Bucharest, Romania

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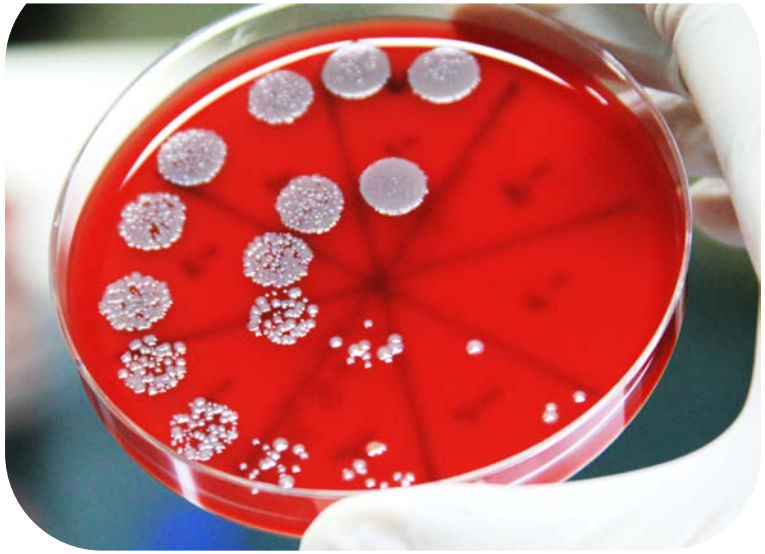
S. aureus



In the beginning…

…there was adhesion

- abiotic surfaces (foreign bodies)
- biotic surfaces (extracellular matrix proteins of the host cell)
- other staphylococcal cells (biofilm accumulation)

- MSCRAMM – microbial surface components recognizing adhesive matrix molecules:
  - proteins binding fibronectin, fibrinogen and collagen

S. aureus biofilms in vitro

Microbial products

- Polysaccharide intercellular adhesin (PIA)[1]
- Teichoic and lipoteichoic acid[1]
- Extracellular DNA
- Serine-aspartate repeat protein C (SdrC)
- MSCRAMMs

- The arlSR TCS modulates the activity of peptidoglican hydrolase
- Bacterial inoculum is important[4]

Substrate characteristics

- *S. aureus* adheres better to polyurethane, compared to titanium

=> titanium is preferred for cardiac implantable electronic devices[5] or prostheses in general[6]

- Higher adhesion to: expanded polytetrafluoroethylene polymer, multifilament meshes, increased filament diameter, increased mesh weight, smaller mean pore size.[7]

[1]Heilmann C. Adv Exp Med Biol 2011;715:105-23.
[2]Barbu EM, et al. Mol Microbiol 2014;94:172-85.
[3]Fournier B, et al. J Bacteriol 2000;182:3955-64.
[4]Sanders DL, et al. Surg Endosc 2013;27:978-85.
[5]Viola GM, et al. Am J Cardiol 2013;111:1764-6.
[6]Perez-Jorge C, et al. J Biomed Mater Res A 2012;100:1696-705.
[7]Sanders D, et al. Hernia 2013;17:779-89.

S. aureus biofilms in vivo (1)

- Intercellular adhesion (ica) locus: **ica-dependent vs. ica-independent biofilm**[1]

- MSCRAMMs:
  - **FnBP A** (fibronectin binding protein A) => adhesion to fibrinogen[2]
  - **ClfA**[3] and **ClfB**[4] (clumping factors A and B) => late adhesion to fibrinogen (after 24 h)

- Aaa autolysin/adhesin => adhesion to **extracellular matrix proteins: fibrinogen, fibronectin, vitronectin**[5]

- **MTA/SAH nucleosidase** induces transcription of autolysins =>**extracellular DNA**[6]

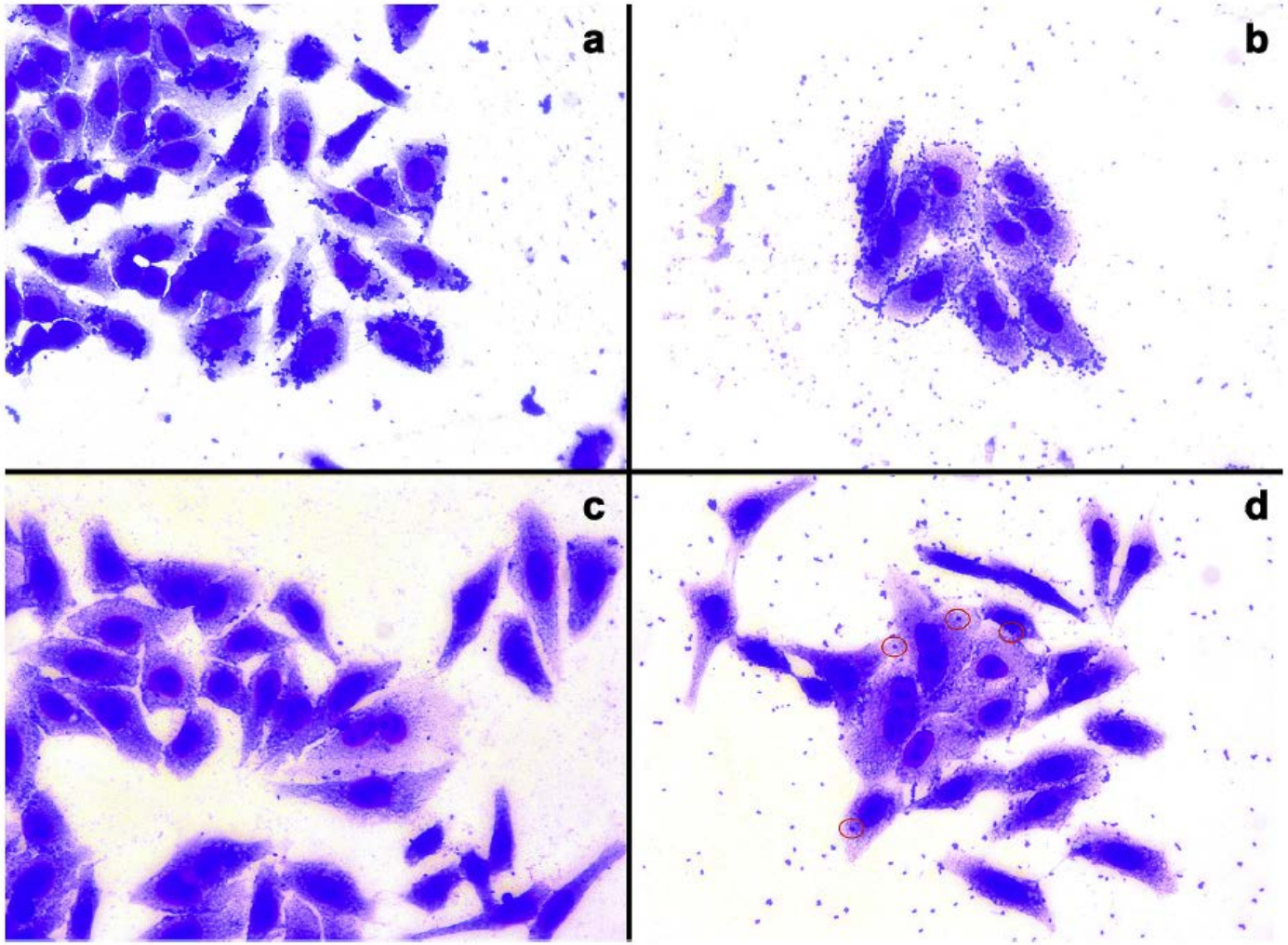
[1]Ferreira FA, et al. J Microbiol Methods 2012;88:393-8.
[2]Geoghegan JA, et al. J Bacteriol 2013;195:2675-83.
[3]Ythier M, et al. Mol Cell Proteomics 2012;11:1123-39.
[4]Atshan SS, et al. Infect Genet Evol 2013;18:106-12.
[5]Hirschhausen N, et al. PLoS One 2012;7:e40353.
[6]Bao Y, et al. Med Microbiol Immunol 2014.

S. aureus biofilms in vivo (2)

- Covalent links between β-hemolysin molecules (sphingomyelinase AND biofilm-ligase)[1.] => **biofilm matrix**
- The SrrAB TCS controls **survival** of static biofilms.[2]
- Upregulation of succinate dehydrogenase (sdhCAB) genes => favors the tricarboxylic acid cycle in **environments with suboptimal nutrient and O_2 supply**[3]
- Bone sialoprotein-binding protein (Bbp) => **osteomyelitis**.[4]

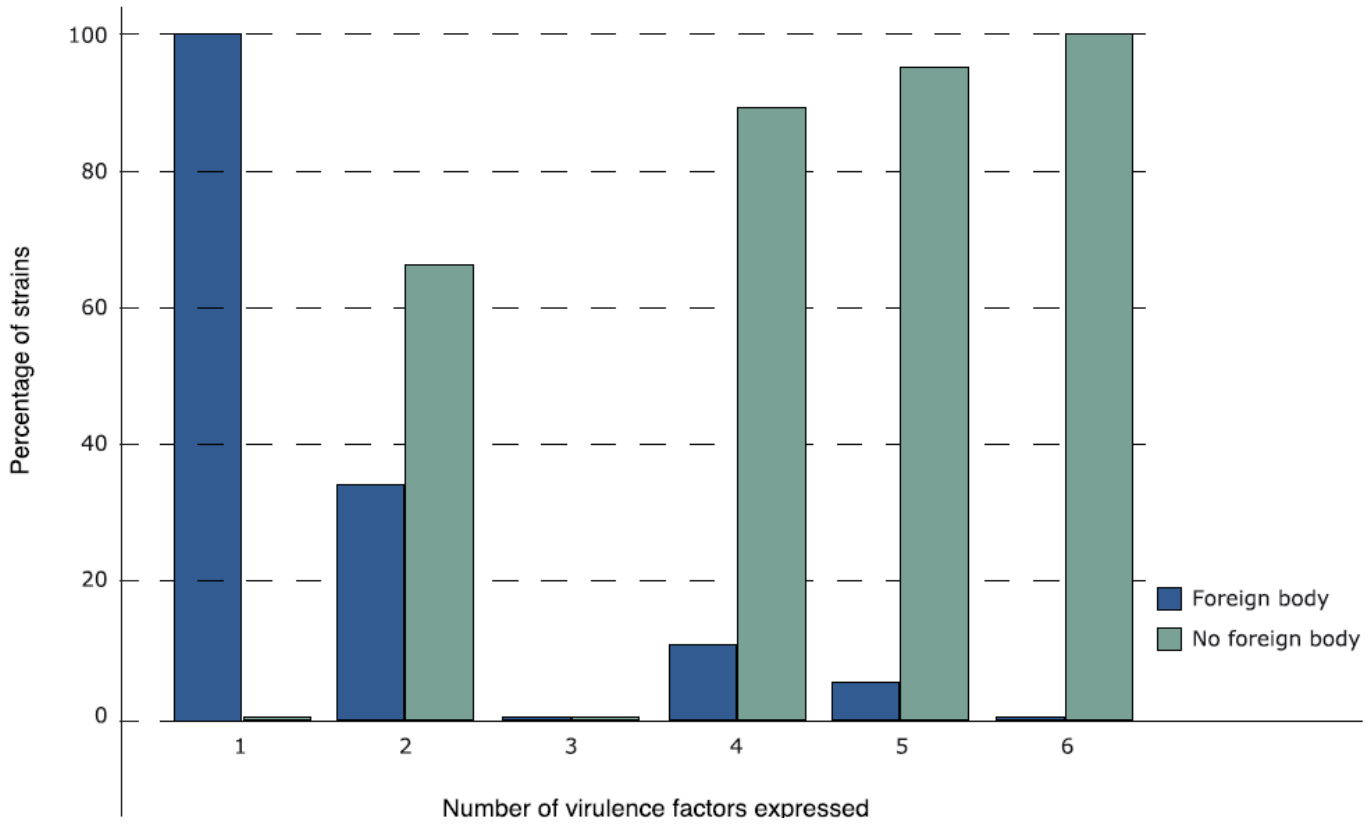
[1]Huseby MJ, et al. Proc Natl Acad Sci U S A 2010;107:14407-12.
[2]Kinkel TL, et al. MBio 2013;4:e00696-13.
[3]Gaupp R, et al. J Bacteriol 2010;192:2385-94.
[4]Vazquez V, et al. J Biol Chem 2011;286:29797-805.

More on staphylococcal pathogenesis…



a. **Aggregative adhesion:** clinical *S. aureus* isolate; high adhesion index (97%); high cluster adhesion (200 cells).
b. **Localized adhesion:** clinical *S. epidermidis* isolate; high adhesion index (96.8%); moderate cluster adhesion (35 cells).
c. **Diffuse adhesion:** commensal *S. epidermidis* strain; low adhesion index (2.6%); low cluster adhesion (6 cells).
d. **Tetrad morphology** (red circles) and localized/diffuse adhesion: clinical *S. epidermidis* isolate; moderate adhesion index (72.3%); low cluster adhesion (6 cells).

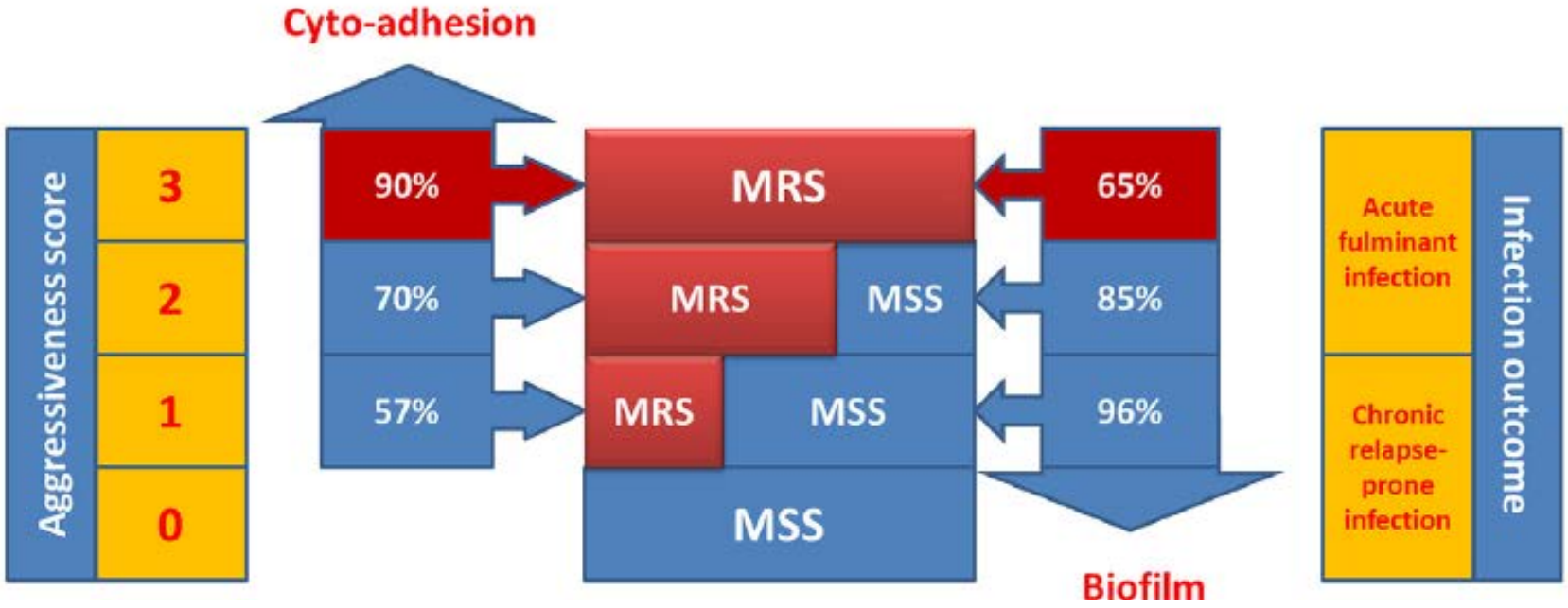
Sandulescu O et al. Microb Pathog 2017;102 45-53.



The overall number of soluble virulence factors expressed was higher in patients without evidence of foreign body while the strains isolated from patients with implanted foreign body expressed significantly fewer virulence factors [P = 0.016, Chi^2= 12.2].

Sandulescu O et al. Microb Pathog 2017;102 45-53.

Staphylococcal aggressiveness score



The staphylococcal aggressiveness score (SAS) is calculated by **allocating one point for the presence of each** of the following bacterial traits (**T-A-M**): **tetrad morphology, aggregative adhesion, methicillin resistance**. The cytoadhesion column illustrates the percentage of strains that have a high adhesion index (>80%) on HEp-2 cell line. The biofilm column presents the percentage of strains that have the capacity to form biofilm. The score can discriminate between chronic biofilm-related relapse-prone infections (scores 0 and 1) and infections with acute fulminant evolution (scores 2 and 3).

Sandulescu O et al. Microb Pathog 2017;102 45-53.

P. aeruginosa biofilms

- Opportunistic germ
- Ventilator-associated pneumonia
- Cystic fibrosis
- Chronic wounds
- Indwelling devices…

P. aeruginosa biofilms (1)

- **Attachment**: adhesins, type IV pili, lipopolysaccharide (LPS)
  - the contact of *P. aeruginosa* to a surface is recognized by the WspA protein
  - WspA creates a signal to produce c-di-GMP
  - c-di-GMP upregulates: CdrA adhesin and EPS (Psl, Pel, alginate)

- **Accumulation and maturation**: changes to adapt to the new mode of life
  - ECM:
    - **EPS**
      - Psl => cell-surface attachment + intercellular interactions, eDNA-Psl web
      - Pel => pellicles at the air-liquid interface; solid surface-associated biofilms; protection against aminoglycosides
      - alginate => retains water and nutrients; blocks antibiotics; immune evasion
    - **eDNA** => eDNA-Psl web; formation of cation gradients
  - structuring
  - creating channels
  - physiological changes

Lee K, et al. J Microbiol Biotechnol. 2017;27:1053-64.

P. aeruginosa biofilms (2)

- **Detachment**:
  - Passive (shear forces):
    - **Sloughing** => detachment of a large portion of a biofilm from the original mass
    - **Erosion** => washout of a small portion of biomass or bacteria from the outer surface
  - Active:
    - **Seed dispersal** => release of single planktonic cells or microcolonies from the center of the biofilm through a complex process:
      - spatial differentiation (motile bacteria in the mushroom cavity; non-motile bacteria at the stalk and walls of the mushroom structure)
      - ECM degradation + autolysis of a biofilm subpopulation
    - May be induced by environmental cues, such as nutrients, oxygen availability, nitric oxide, pH, chemicals.

Lee K, et al. J Microbiol Biotechnol. 2017;27:1053-64.

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Biofilm active agents – types of agents and mechanisms of action –

Oana Săndulescu, MD, PhD

Associate Professor, Infectious Diseases

Carol Davila University of Medicine and Pharmacy, Bucharest, Romania

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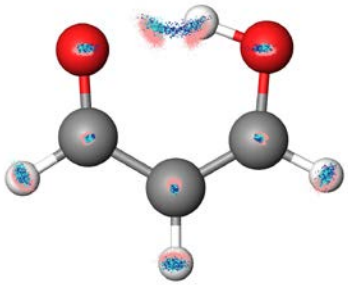
Types of biofilm active agents

- Natural products

- Antimicrobial agents

- Synthetic peptides



- Materials and devices

Săndulescu O, et al. Anti-biofilm activity of viruses, bacteria, fungi and lichens – mechanisms and impact on clinical practice. In press.

Natural anti-biofilm agents

- **Viruses**:
  - bacteriophages => destroy bacterial cells; produce enzymes to circumvent bacteria's ability to form biofilms

- **Bacteria**: bioactive peptides; secondary metabolites:
  - lytic action
  - inhibiting the formation of extracellular polymeric substances

- **Fungi**: clinically-relevant fungi; lichen-associated fungi:
  - cell wall components
  - secondary metabolites

Săndulescu O, et al. Anti-biofilm activity of viruses, bacteria, fungi and lichens – mechanisms and impact on clinical practice. In press.

Bacteriophages

- Lysis of bacterials cells = nobody left to form a biofilm
  - Correct?
  - Incorrect?

- However, the clinical scenario is more complex:
  - Gram-negative germs: lysis = release of endotoxin (lipid A component)[1,2]
  - => exaggerated proinflammatory cytokine responses
  - *E. coli* can display on its surface up to 10^6 lipid A residues, and induce a strong host response[3]

- => Use of lysis-deficient bacteriophages[2]

[1]Brzozowska E, et al. Sci Rep. 2017;7:18048.
[2]Colavecchio A, et al. Microbiol Spectr. 2017;5(3).
[3]Ramachandran G. Virulence. 2014;5:213-8.

Bacteriophages in clinical practice

- Georgia – standalone treatment
- Poland – last resort therapy
- Romania – last resort add-on to antimicrobial therapy
- UK – veterinary use
- …?

Phage-encoded enzymes (1)

- Depolymerases[1] => activity against carbohydrates found in **capsular polysaccharides** and in **EPS**:
  - O-glycosyl hydrolases (sialidases or neuraminidases, levanases, peptidases; xylosidases, dextranases, rhamnosidases)
  - polysaccharide lyases (hyaluronate lyases, alginate lyases, pectin/pectate lyases)
  - other types of enzymes (lipases)

- Alginate lyase => reduction of exopolysaccharides produced by *P. aeruginosa* mucoid strains from patients with CF[2,3]
- CHAPK murein peptidase => inhibits biofilm formation and disrupts mature MRSA biofilm.[4]

[1]Pires DP, et al. Appl Microbiol Biotechnol. 2016;100:2141-51.
[2]Glonti T, et al. J Appl Microbiol. 2010;108:695-702.
[3]Latka A, et al. Appl Microbiol Biotechnol. 2017;101:3103-19.
[4]Keary R, et al. Curr Protein Pept Sci. 2016;17:183-90.

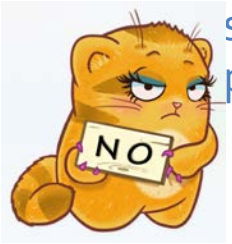
Phage-encoded enzymes (2)

- **Lysozymes**: Cpl-1, Cpl-7 => active on *S. pneumoniae*, *S. pseudopneumoniae*, *S. oralis* 14-16-hour biofilms.[1]

- **Endolysins** = peptidoglycan hydrolases: lytic activity (hydrolysis of peptidoglycan layers): MR-10, LysK, lysostaphin, Twort, phiSH2,[2] LysH5,[3] SAP-2, PlyGRCS, etc.

  - **management of Gram-positive** biofilm-related infections

  - structural characteristics of **Gram-negative** germs make them less susceptible to endolysins: their outer membrane efficiently covers the peptidoglycan layer



    - **pre-treatment with outer membrane permeabilizers**: chelators (EDTA), polycationic agents such as polymyxins, aminoglycosides or lysine polymers

[1]Domenech M, et al. Antimicrob Agents Chemother. 2011;55:4144-8.
[2]Schmelcher M, et al. J Antimicrob Chemother. 2015;70:1453-65.
[3]Gutierrez D, et al. PLoS One. 2014;9:e107307.

Phage-encoded proteins

Tail tubular proteins:

- TTPAgp31 from *Klebsiella pneumoniae* bacteriophage KP32
  - Degrades multiple types of *K. pneumoniae* polysaccharides through an α-1,4-glucosidase activity:
    - capsular polysaccharides
    - cell-free (slime) polysaccharides
    - lipopolysaccharides
  - Decreases biofilm biomass by 80% for *K. pneumoniae*, 50% for *S. aureus* and 60% for *E. faecalis*

- TTPAgp44 from *K. pneumoniae* bacteriophage KP34:
  - Hydrolyzes *E. faecium* capsular polysaccharides through a glucohydrolase-like activity
  - Reduces the bacterial biomass by 80% for *E. faecium*, 40% for *P. aeruginosa* and 40% for *Bacillus subtilis*.

Brzozowska E, et al. Sci Rep. 2017;7:18048.

Bacterial products

- In natural environments, bacteria often come into contact with each other, and they can display a complex range of interactions:
  - collaborating within microbial consortia
  - competing with each other for scavenging resources
  - directly attacking each other by synthetizing specific **molecules, bioactive peptides,** or by **secondary metabolites**.

Bacterial products (1)

- LytA (N-acetylmuramoyl-l-alanine amidase)
  - = pneumococcal autolysin
  - synergic with the Cpl-1 bacteriophage-derived lysozyme[1]

- Secondary metabolites of *Bacillus aneurinolyticus*, *Bacillus brevis*:
  - tyrocidines TrcA, TrcB, and gramicidin S inhibit biofilm by *C. albicans*
  - synergic with caspofungin and amphotericin B[2]

- D-amino acids produced by mature (5-8 days) biofilm *Bacillus subtilis:*[3]
  - A mixture of D-leucine, D-methionine, D-tyrosine and D-tryptophan is incorporated into the cell wall in the 3rd day of growth. Once incorporated, they impair the anchoring into of amyloid fibers the cell wall.
  - Active on *B. subtilis*, *S. aureus* and *P. aeruginosa*

[1]Chopra S, et al. Appl Microbiol Biotechnol. 2015;99:3201-10.
[2]Troskie AM, et al. Antimicrob Agents Chemother. 2014;58:3697-707.
[3]Kolodkin-Gal I, et al. Science. 2010;328:627-9.

Bacterial products (2)

- Pyoverdine produced by *P. aeruginosa* acts as a siderophore, inducing iron starvation and inhibiting biofilm by *A. fumigatus*.[1]

- Acyldepsipeptides (ADEPs) produced by *Streptomyces hawaiiensis:*
  - A semi-synthetic derivative, ADEP4, binds to the ClpP protease and **activates proteolysis** => **destruction of bacterial cells**, both metabolically active and inactive, and **specifically bacterial persisters**
  - Its association with rifampin **fully eradicated *S. aureus* biofilm** in vitro and in a murine thigh infection model[2]

[1]Sass G, et al. J Bacteriol. 2018;200(1).
[2]Conlon BP, et al. Nature. 2013;503:365-70.

Oral microbiota

Lactobacillus spp:

- **inhibit biofilm formation by *Streptococcus mutans*** (by inhibiting production of exopolysaccharide from sucrose)[1,2]
- **inhibit biofilm formation by *C. albicans*** (through their production of exometabolites and organic acids)[3]
- **inhibit *C. albicans* yeast-to-hyphae differentiation**[4]
- also **active on mixed** *S. mutans* + *C. albicans* **biofilms**
- **produce post-biotics:** bacteriocins able to inhibit biofilm formation by *P. aeruginosa* PAO-1[5] and *S. aureus*[6]

[1]Ahn KB, et al. PLoS One. 2018;13:e0192694.
[2]Wasfi R, et al. J Cell Mol Med. 2018.
[3]Rossoni RD, et al. Biofouling. 2018;34:212-25.
[4]Matsubara VH, et al. Appl Microbiol Biotechnol. 2016;100:6415-26.
[5]Sharma V, et al. Folia Microbiol (Praha). 2018;63:181-90.
[6]Okuda K, et al. Antimicrob Agents Chemother. 2013;57:5572-9

Other microbiota

Bifidobacteria:

- **inhibit biofilm formation by enterohemorrhagic *E. coli* O157:H7**
- **attenuate its virulence** in a *Caenorhabditis elegans* model[1]

[1]Kim Y, et al. Anaerobe. 2012;18:539-45.

Silver nanoparticles

Silver nanoparticles biosynthesized from:

- ***Spirulina platensis***
  - functionalization of Foley catheters in combination with amikacin and nitrofurantoin
  - murine model of UTI
  - **complete inhibition of colonization and biofilm formation by uropathogenic *E. coli*** for 14 days[1]

- ***Streptomyces calidiresistens***
  - **inhibit biofilm formation by *S. aureus*, *E. coli* and *C. albicans*[2]**

[1]Mala R, et al. IET Nanobiotechnol. 2017;11:612-20.
[2]Wypij M, et al. J Appl Microbiol. 2018.

Fungal products as anti-biofilm agents

- Cell wall components
- Secondary metabolites

- *Penicillium* spp. products inhibit *S. aureus* biofilms:
  - dipeptide cis-cyclo(Leucyl-Tyrosyl)[1]
  - norlichexanthone[2]

- *Penicillium* spp. secondary metabolites disrupt *C. albicans* biofilms:
  - shearinines (also synergic with amphotericin B)[3]

[1]Scopel M, et al. Bioorg Med Chem Lett. 2013;23:624-6.
[2]Baldry M, PLoS One. 2016;11:e0168305.
[3]You J, et al. ACS Chem Biol. 2013;8:840-8.

Fungal products as anti-biofilm agents

Anti-biofilm agents that <u>do not display antimicrobial activity</u>:

- **Mannoprotein** = surfactant extracted from *Saccharomyces cerevisiae* cell wall
  - No antimicrobial activity on *S. aureus* or *S. epidermidis*.
  - It is active on biofilm by influencing cell surface hydrophobicity.[1]

- **Patulin** and **emodin** = metabolites from *Plectosphaerella cucumerina*
  - No antimicrobial activity on *P. aeruginosa* PAO1.
  - Inhibits the production of virulence factors: protease, elastase and pyocyanin.[2]

[1]Walencka E, et al. Z Naturforsch C. 2007;62:433-8.
[2]Zhou J, et al. Front Microbiol. 2017;8:769.

Secondary metabolites of licheni-associated fungi

Usnic acid

- **Inhibits biofilms** by most group A streptococci, *S. aureus* from CF, *S. epidermidis*, *C. albicans*, *C. orthopsilosis*, but not by *C. krusei*

- **Targeted delivery systems**:
  - loaded magnetic nanoparticles
  - loaded carboxylated poly(L-lactide) microparticles
  - coated magnetic polylactic-co-glycolic acid-polyvinyl alcohol (PLGA-PVA) microsphere thin films
  - surface coated zirconium dioxide bearing and barium sulfate bearing bone cement

Săndulescu O, et al. Anti-biofilm activity of viruses, bacteria, fungi and lichens – mechanisms and impact on clinical practice. In press.

Antimicrobials as anti-biofilm agents

Săndulescu O, et al. Anti-biofilm agents. In: Shifmann, MA, ed. Recent Clinical Techniques, Results, and Research in Wounds. Cham, Switzerland: Springer, 2017. doi: 10.1007/15695_2017_4.

S. aureus biofilms – betalactams and clindamycin

- **Antimicrobial associations:**
  - Severe infections: anti-MRSA + wide spectrum betalactam drug
  - Microbial consortia: CF, wounds, etc.

- **Paradoxical effect on *S. aureus* biofilms!**
  - Sub-MIC levels of **betalactams** in the infectious site **increase biofilm formation**[1]
  - Sub-MIC levels of **clindamycin** in the infectious site **increase biofilm formation**[2]

- While betalactams (except for C5G) are not used in the treatment of staphylococcal infections, **clindamycin** is
  - => clindamycin should be administered in high doses when used in *S. aureus* infections, to ensure that the MIC is obtained in the infectious site.
  - => clindamycin should not be considered effective on mature biofilm regardless of the dose used.

[1]Ng M, et al. Dose Response. 2014;12:152-61.
[2]Schilcher K, et al. Antimicrob Agents Chemother. 2016;60:5957-67.

S. aureus biofilms – glycopeptides, lipopeptides, lipoglycopeptides

- **Glycopeptides** are not useful in the treatment of biofilm-associated infections:
  - **Vancomycin**: limited anti-biofilm effect (some inhibition of biofilm formation)
  - **Teicoplanin**: slightly better.

- **Lipopeptides**
  - **Daptomycin** may be useful in prevention of biofilm formation, or treatment of **recent** staphylococcal biofilm-driven infections; targeted local delivery by a nanocage-based system

- **Lipoglycopeptides**
  - Oritavancin < dalbavancin < telavancin

Săndulescu O, et al. Anti-biofilm agents. In: Shifmann, MA, ed. Recent Clinical Techniques, Results, and Research in Wounds. Cham, Switzerland: Springer, 2017. doi: 10.1007/15695_2017_4.

S. aureus biofilms – macrolides, oxazolidinones

Clarithromycin

- Not used for treating staphylococcal infections
- May inhibit *S. aureus* biofilm formation by **decreasing MRSA glycocalyx production**, its effect being independent of its antimicrobial spectrum
- Synergistic activity on MRSA biofilm eradication when administered with vancomycin, moxifloxacin or daptomycin

Oxazolidinones:

- Linezolid: inhibition of biofilm formation; needs **10 × MIC** to eradicate mature biofilm
- Linezolid < tedizolid

Săndulescu O, et al. Anti-biofilm agents. In: Shifmann, MA, ed. Recent Clinical Techniques, Results, and Research in Wounds. Cham, Switzerland: Springer, 2017. doi: 10.1007/15695_2017_4.

P. aeruginosa biofilms – betalactams

Ceftazidime:

- Active on biofilms at 16-64 × MIC => catheter antimicrobial lock
- Induction of betalactamase expression in mature biofilm[1]

Meropenem:

- Some decrease in mature biofilm
- Biofilm inhibitory concentration 4-16 × MIC
- Bactericidal only to the outer biofilm layer[2]
- Strong(er) induction of betalactamase expression in mature biofilm[1]
- The addition of **dexamethasone inhibits its anti-biofilm effect**[3]

[1]Bowler LL, et al. Antimicrob Agents Chemother. 2012;56:4976-9.
[2]Haagensen JA, et al. Antimicrob Agents Chemother. 2015;59:4074-81.
[3]Rodrigues A, et al. J Adv Res. 2017;8:55-61.

P. aeruginosa biofilms – fosfomycin

- Fosfomycin **lacks anti-biofilm activity when administered alone**

- It displays **synergy with aminoglycosides**:

  tobramycin < netilmicin < gentamicin/isepamicin < amikacin

- It displays **synergy with fluoroquinolones**:

  ciprofloxacin < levofloxacin; or ciprofloxacin = levofloxacin.

Săndulescu O, et al. Anti-biofilm agents. In: Shifmann, MA, ed. Recent Clinical Techniques, Results, and Research in Wounds. Cham, Switzerland: Springer, 2017. doi: 10.1007/15695_2017_4.

P. aeruginosa biofilms – colistin

- Colistin can be useful in preventing biofilm formation[1]
- Its activity on mature biofilm is comparable to that of meropenem
- It can eradicate mature *P. aeruginosa* biofilm mainly when used as **antimicrobial lock agent.**[2]
- It is **active on sessile cells exhibiting low metabolic activity**
- It can be successfully associated to ciprofloxacin, which targets metabolically-active cells.[3]
- Its activity on mature biofilm is concentration-dependent. This is **only true when administered with a correct loading dose.** The effect is lost when treatment is started with lower concentrations leading to phenotypic tolerance.[4,5]

[1]Mohamed NM, et al. Microb Drug Resist. 2011;17:489-95.
[2]Ozbek B, et al. J Chemother. 2016;28:20-4.
[3]Pamp SJ, et al. Mol Microbiol. 2008;68:223-40.
[4]Haagensen JA, et al. J Bacteriol. 2007;189:28-37.
[5]Streinu-Cercel A. Germs. 2014;4:7-8.

P. aeruginosa biofilms – tigecycline

- Tigecycline has variable efficiency in preventing biofilm formation by Gram-negative germs
- It is **active** mostly **on *Escherichia coli*** and ***Acinetobacter baumannii***
- It fails to exhibit significant activity against *P. aeruginosa*

Mohamed NM, et al. Microb Drug Resist. 2011;17:489-95.

Fluoroquinolones as anti-biofilm agents

S. aureus biofilms

Ciprofloxacin

- Bactericidal only at a minimum biofilm eradication concentration (MBEC) **256 × MIC = clinically unachievable!**[1,2]
- Could be considered for local administration with conditioned release from cement, loaded particles, beads, or specific wound dressings

Levofloxacin: same as ciprofloxacin.

Moxifloxacin: no activity on biofilms.

P. aeruginosa biofilms

Ciprofloxacin

- **Good anti-biofilm activity!**
- Active on *P. aeruginosa* biofilms at concentrations well within what can be achieved in clinical practice
- Only active on metabolically active sessile cells
- Association with membrane-acting agents (colistin) can jointly target cells with high and low metabolic activity

Levofloxacin: same as ciprofloxacin.

Moxifloxacin: no activity on biofilms.

[1]Thomas N, et al. J Pharm Sci. 2016;105:3115-22.
[2]Molina-Manso D, et al. Int J Antimicrob Agents. 2013;41:521-3.

Rifampin as anti-biofilm agent

S. aureus biofilms

- Useful in the treatment of biofilm-related infections
- Synergy with: ceftaroline, ceftobiprole, daptomycin, clarithromycin, linezolid, tigecycline
- No synergy with: vancomycin, fosfomycin
- **Should never be used alone! –** resistance occurs within 3 days of monotherapy

P. aeruginosa biofilms

- Not used as antimicrobial treatment
- Can be active as associated agent on 20-hour mature *P. aeruginosa* biofilms, when reaching local concentrations twice its MIC.[2]

[1]Tang HJ, et al. J Antimicrob Chemother. 2012;67:944-50.
[2]Moskowitz SM, et al. J Clin Microbiol. 2004;42:1915-22.

Aminoglycosides as anti-biofilm agents

S. aureus biofilms

- **Gentamicin**: good activity on mature *S. aureus* biofilm[1]
- Synergy with tigecycline, but not with ceftobiprole
- Oto- and nephro- toxicity!
- Should not be used as monotherapy

P. aeruginosa biofilms

- **Gentamicin:** partially suppresses biofilm growth for 96 hours; fails to completely inhibit biofilm growth at MIC
- The addition of **dexamethasone inhibits its anti-biofilm effect**[2]
- **Amikacin: potent anti-biofilm activity**

Moxifloxacin < levofloxacin/gentamicin < ciprofloxacin/tobramycin[3]

[1]van der Horst AS, et al. J Orthop Res. 2015;33:1320-6.
[2]Rodrigues A, et al. J Adv Res. 2017;8:55-61.
[3]Elkhatib W, et al. Antibiotics (Basel). 2014;3:64-84.

Antimicrobial associations – *S. aureus*

	Oxacillin	Ceftaroline	Ceftobiprole	Vancomycin	Teicoplanin	Daptomycin	Telavancin	Dalbavancin	Clarithromycin	Telithromycin	Ciprofloxacin	Levofloxacin	Moxifloxacin	Rifampin	Linezolid	Tedizolid	Gentamicin	Doxycycline	Tigecycline	Clindamycin	Co-trimoxazole	Quinupristin-dalfopristin	Fosfomycin
Oxacillin	N/A																						
Ceftaroline		N/A		Y [57]		Y [57]								Y [57]									
Ceftobiprole			N/A			N [29]								Y [29]			N [29]						
Vancomycin		Y [57]		N/A		N [58]			Y [12]					Y [58] N [29]	N [58]								Y [31]
Teicoplanin					N/A																		Y [31]
Daptomycin		Y [57]	N [29]	N [58]		N/A			Y [43.55] N [36]					Y [36.58]	N [58]								
Telavancin							N/A																
Dalbavancin								N/A															
Clarithromycin				Y [12]		Y [43.55] N [36]			N/A				Y* [43]	Y [46]									
Telithromycin										N/A													
Ciprofloxacin											N/A												
Levofloxacin												N/A											
Moxifloxacin									Y* [43]				N/A										
Rifampin		Y [57]	Y [29]	Y [58] N [29]		Y [36.58]			Y [46]					N/A	Y [58]				Y [47.58]				N [31]
Linezolid				N [58]		N [58]								Y [58]	N/A								Y [31]
Tedizolid																N/A							
Gentamicin			N [29]														N/A		Y [31]				
Doxycycline																		N/A					
Tigecycline														Y [47.58]			Y [47]		N/A				
Clindamycin																				N/A			
Co-trimoxazole																					N/A		
Quinupristin-dalfopristin																						N/A	
Fosfomycin				Y [31]	Y [31]									N [31]	Y [31]								N/A

*Moxifloxacin lacks anti-biofilm effect on *S. aureus* [43] and clarithromycin lacks efficacy on MRSA and therefore their association does not appear to be a suitable treatment option

N/A not applicable, *Yellow* data for MSSA only, *Orange* data for MRSA only, *Green* data for MSSA and MRSA

Săndulescu O, et al. Anti-biofilm agents. In: Shifmann, MA, ed. Recent Clinical Techniques, Results, and Research in Wounds. Cham, Switzerland: Springer, 2017. doi: 10.1007/15695_2017_4.

Antimicrobial associations – *P. aeruginosa*

Table 4 Efficacy of antimicrobial associations in the treatment of *P. aeruginosa* biofilm-driven infections

	Carbenicillin	Ticarcillin	Piperacillin-tazobactam	Ceftazidime	Cefoperazone	Cefepime/Cefpirome	Ceftobiprole	Meropenem	Imipenem-cilastatin	Doripenem	Aztreonam	Ciprofloxacin	Levofloxacin	Rifampin	Amikacin	Gentamicin	Doxycycline	Co-trimoxazole	Fosfomycin	Colistin
Carbenicillin	N/A																			
Ticarcillin		N/A													N [65]					
Piperacillin-tazobactam			N/A																	
Ceftazidime				N/A																Y [65]
Cefoperazone					N/A															
Cefepime/cefpirome						N/A														
Ceftobiprole							N/A													
Meropenem								N/A				Y [65]						N [65]		
Imipenem-cilastatin									N/A											
Doripenem										N/A										Y [77]
Aztreonam											N/A									
Ciprofloxacin								Y [65]				N/A			N [65]			N [65]	Y [81,93]	Y [65,82]
Levofloxacin													N/A						Y [81,93]	
Rifampin														N/A						
Amikacin		N [65]										N [65]			N/A				Y [86]	
Gentamicin																N/A			Y [86]	
Doxycycline																	N/A			
Co-trimoxazole								N [65]				N [65]						N/A		Y [65]
Fosfomycin												Y [81,93]	Y [81,93]		Y [86]	Y [86]			N/A	
Colistin				Y [65]						Y [77]		Y [65,82]						Y [65]		N/A

N/A not applicable

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Thank you!