Candida albicans biofilm: Causes and solutions

By

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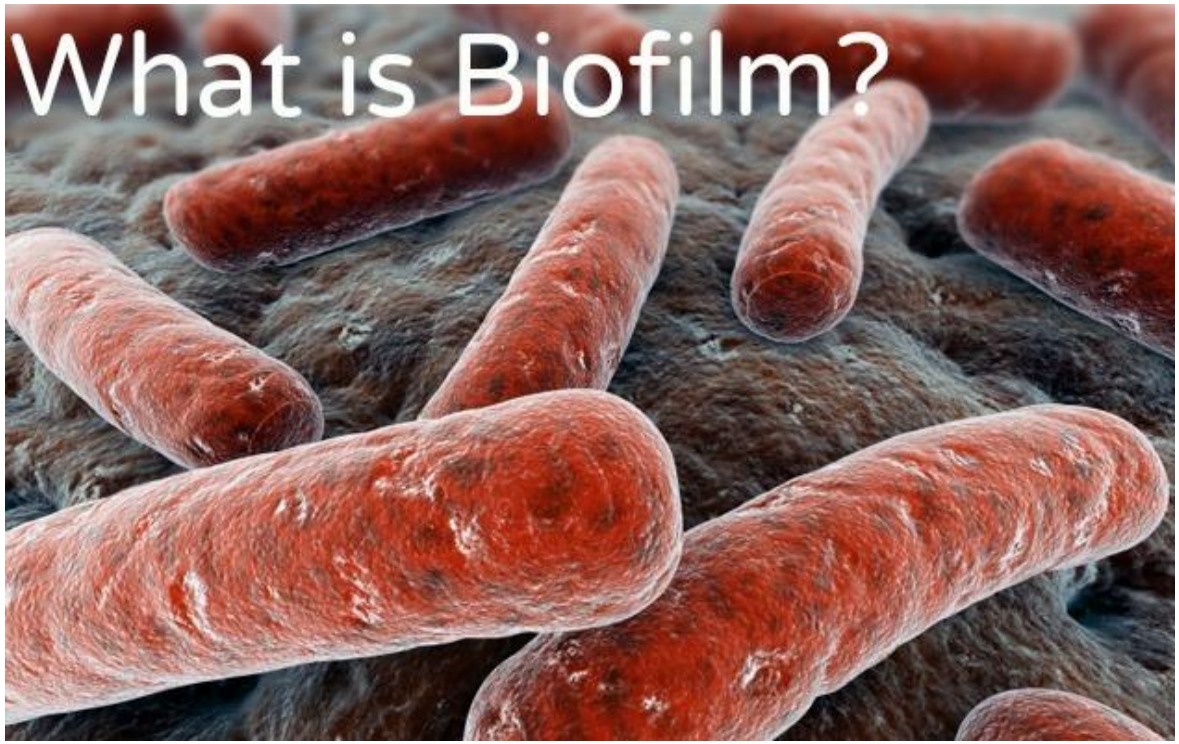
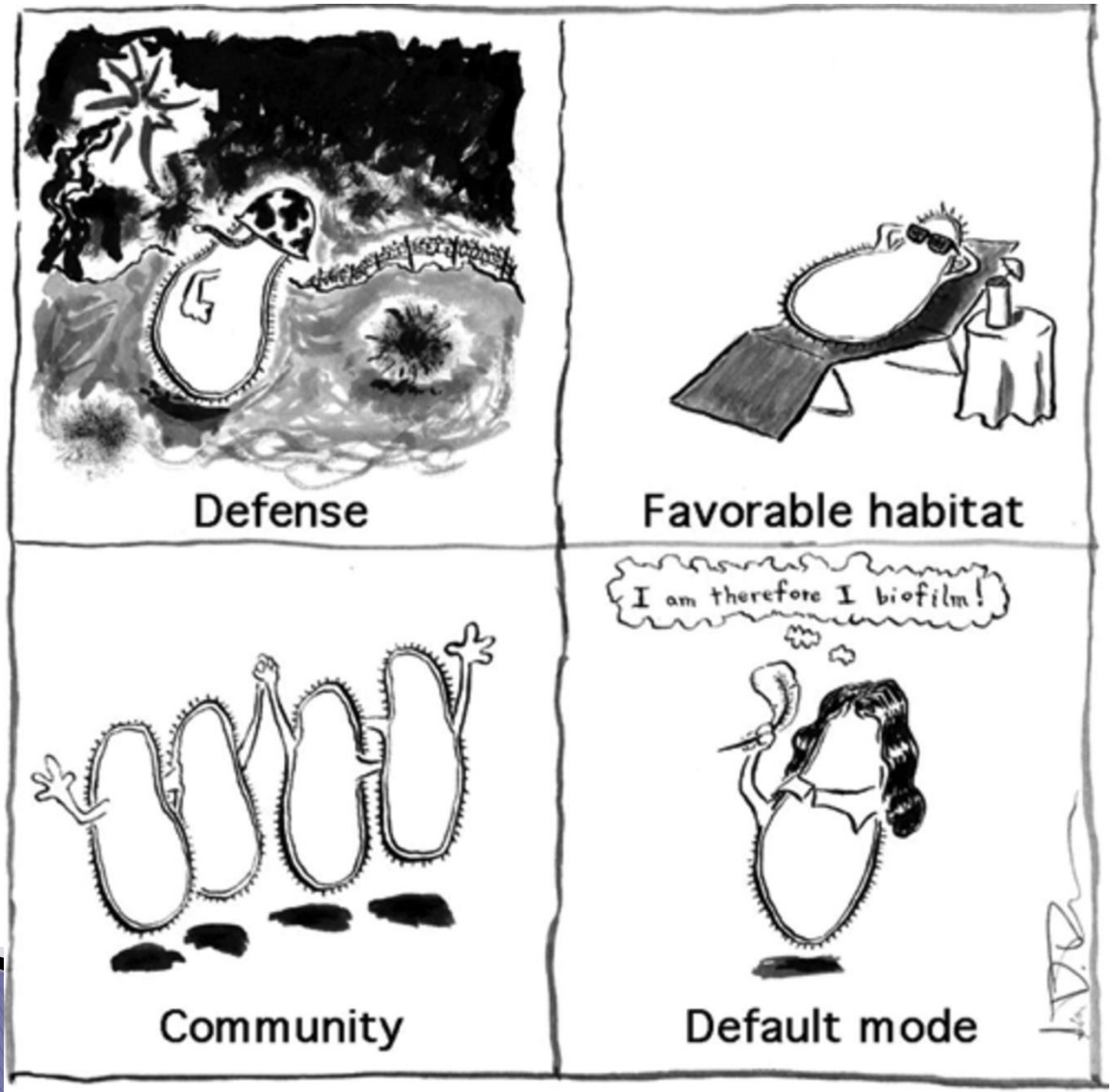
Overview

- What is a biofilm?
- Why microorganisms form biofilm?
- Types of Microorganisms forming Biofilm
- Where are Biofilms Found?
- Stages of development of a Biofilm
- Mechanisms of Antimicrobial Resistance in Biofilms
- Detection of biofilm producer microorganism
- Antifungal therapy of *Candida albicans* biofilms: Past, present and future



- Free Living (planktonic) or biofilm
- Majority of micro-organisms rather live as biofilms than as planktonic cells.

Why microorganisms form biofilm ?



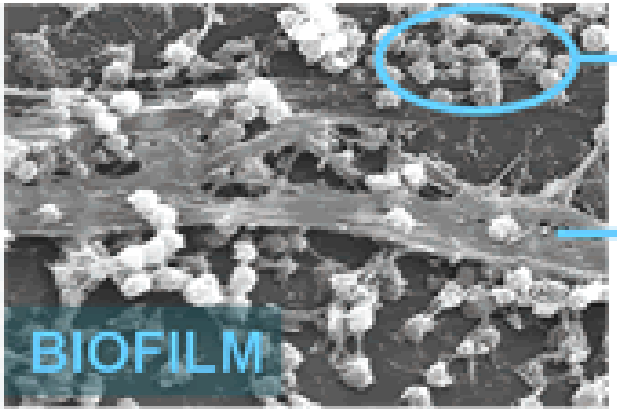
Antibiotic resitance of biofilm in comparison to planktonic (free cells)

Antibiotic	Planktonic MIC_{90} (μg/mL)	Planktonic MIC_{50} (μg/mL)	Biofilm MIC_{50} (μg/mL)	Ratio biofilm MIC_{50}/planktonic MIC_{50}
Rifampicin	3.9×10^{-3}	2.0×10^{-3}	31×10^{-3}	16
Penicillin G	7.8×10^{-3}	$<0.12 \times 10^{-3}$	130×10^{-3}	1000
Oxacillin	63×10^{-3}	0.24×10^{-3}	250×10^{-3}	1000
Fusidic acid	250×10^{-3}	63×10^{-3}	63×10^{-3}	1
Vancomycin	2	16×10^{-3}	1	64
Streptomycin	16	8	64	8
Polymyxin B	32	1	>1024	1000
Amphotericin B	>1024	>1024	>1024	1

MICs of the antibiotic compounds tested on *S. aureus biofilms* determined by crystal violet staining, and the ratio of the MIC of biofilms compared with the MIC of planktonic bacteria

Types of Microorganisms forming Biofilm

- Bacteria
- Fungi
- Yeast (Black yeast)
- Archeae
- Lichens
- Consortium



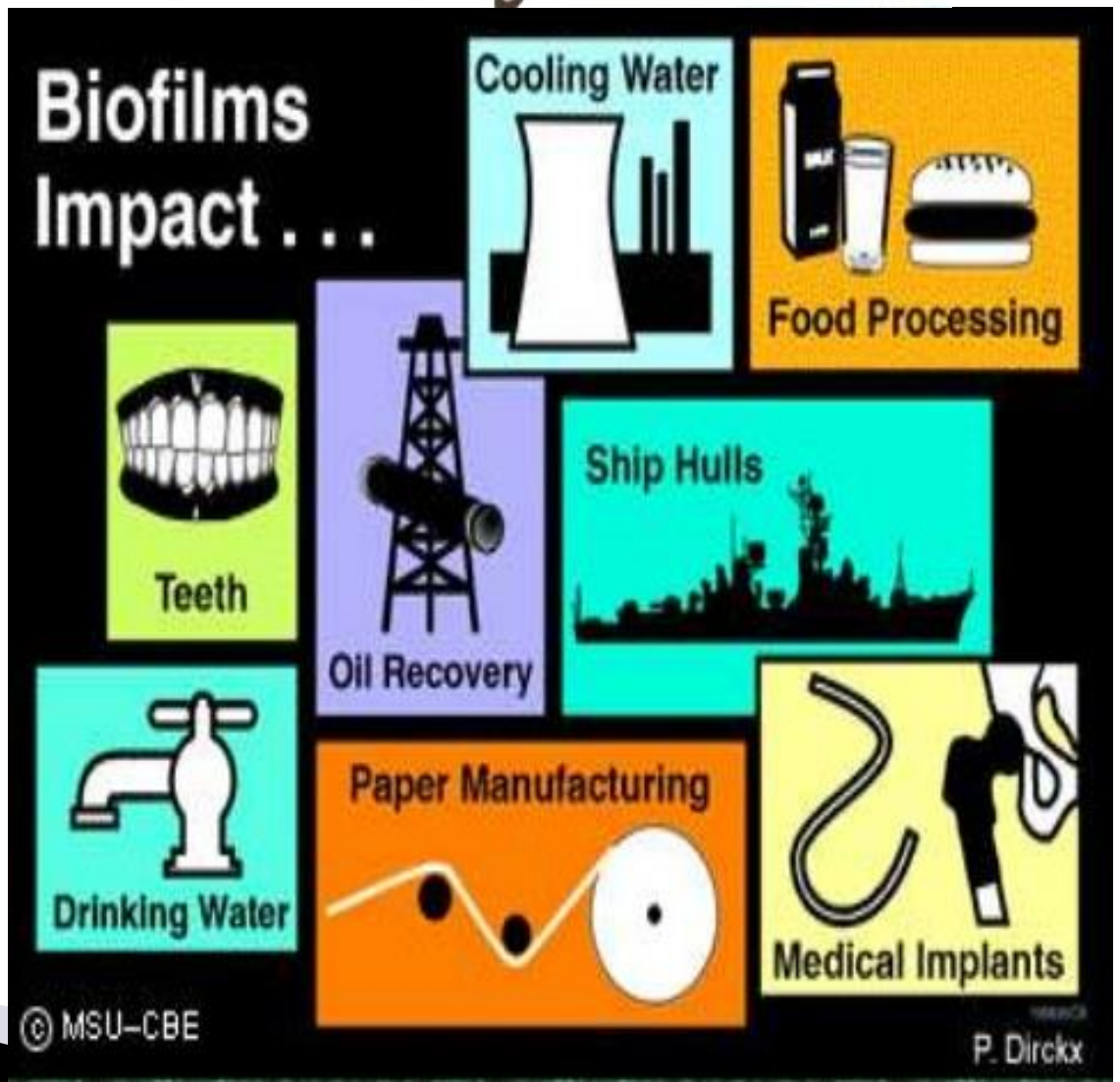
Micro-organisms
(bacteria, fungi, archeae, algeae…)

Biofilm binding matrix
(exopolysaccharides, proteïns…)

Where are Biofilms Found?

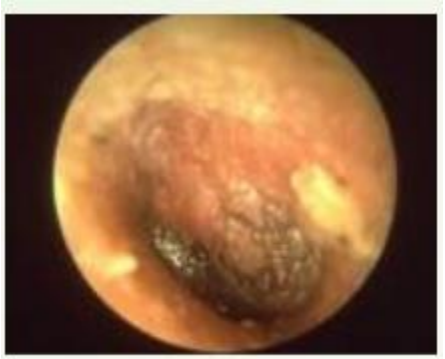
Biofilms are everywhere

1- Nature
2- Industry
3- Organ related infections
4-Indwelling medical devices

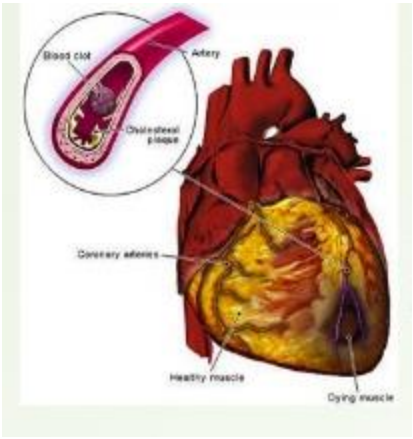


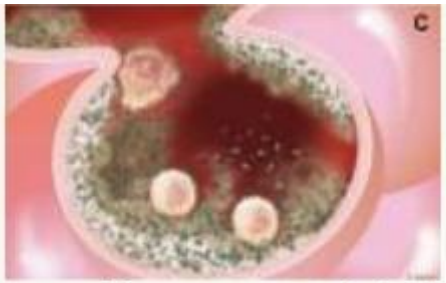
Organs biofilm related infections

1- Otitis media
2- Sinusitis
3- Dental caries, Gingivitis
4- Endocarditis
5- Lung diseases
6- UTI
7- Cystic fibrosis

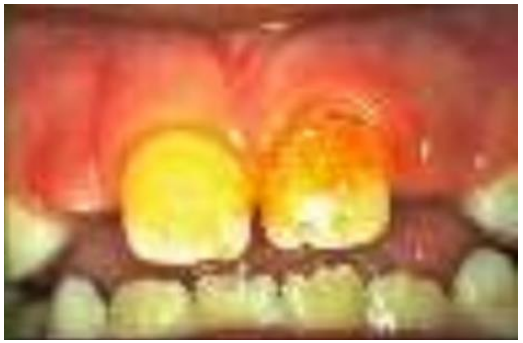


Otitis Media Biofilm

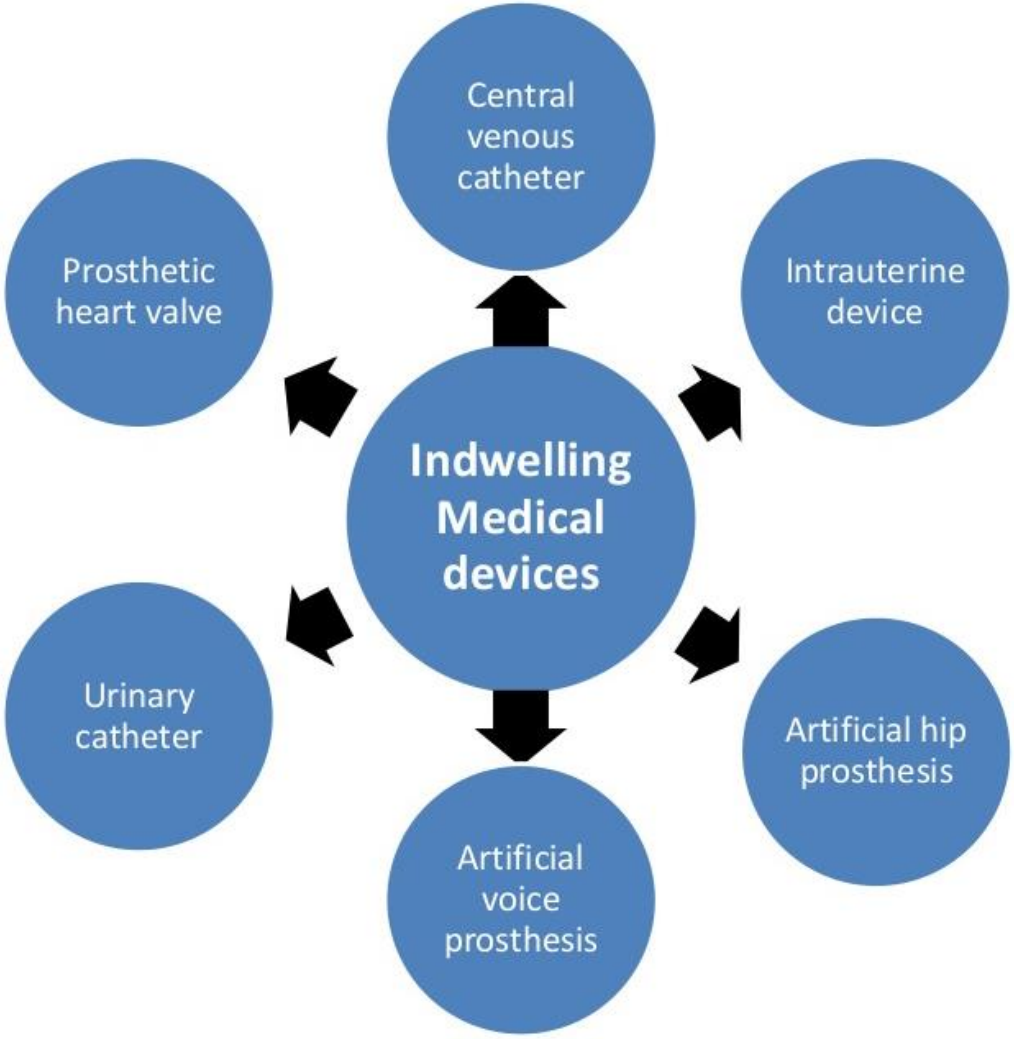




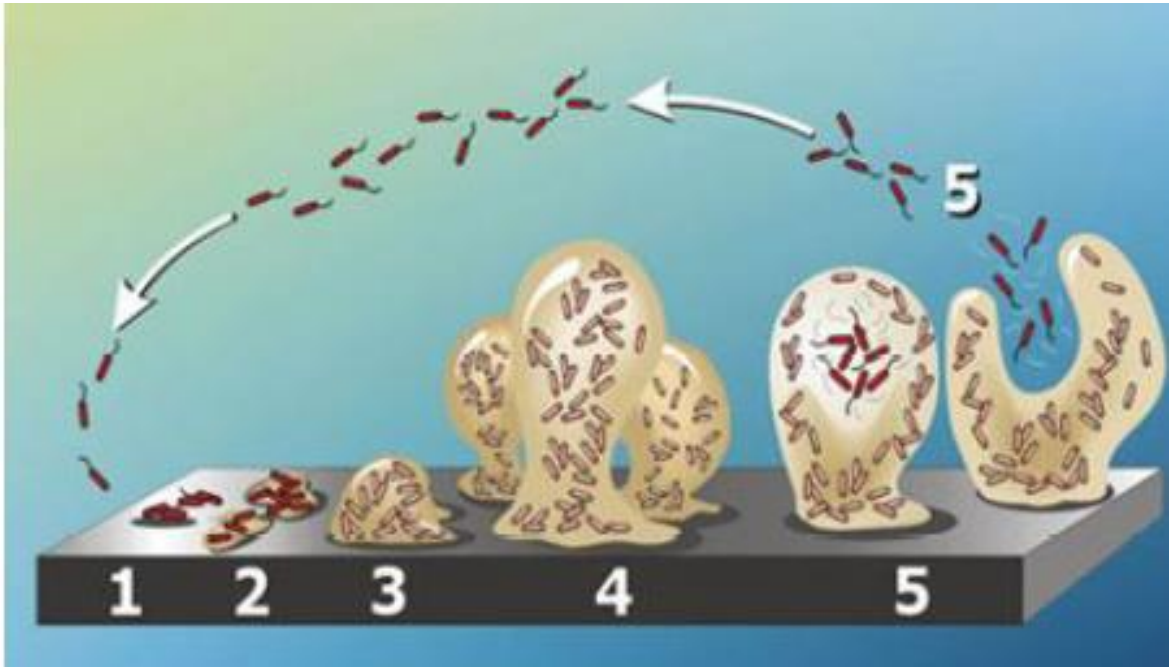
Pseudomonas A. biofilm inside the alveoli in the lung



Biofilms on Indwelling Medical Devices (IMD)

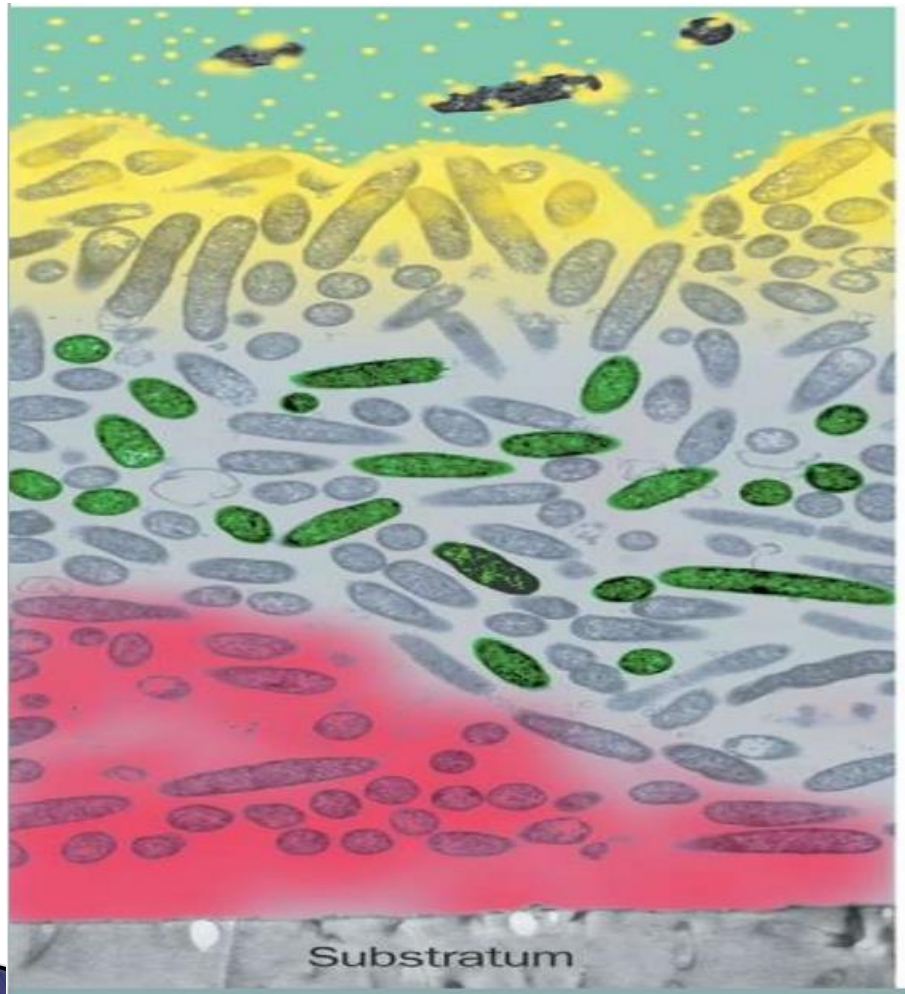


STAGES OF DEVELOPMENT OF A BIOFILM



Stage 1: initial reversible attachment.
Stage 2: irreversible" attachment (production of EPS resulting in more firmly adhered)
Stage 3: early development of biofilm architecture.
Stage 4: maturation of biofilm architecture.
Stage 5: dispersion of cells from the biofilm into the surrounding environment.

Mechanisms of Antimicrobial Resistance in Biofilms



Slow penetration

Antibiotic (yellow) may fail to penetrate beyond the surface layers of the biofilm

Resistant phenotype

Some of the bacteria may differentiate into a protected phenotypic state (green)

Altered microenvironment

In zones of nutrient depletion or waste product accumulation (red), antibiotic action may be antagonised

Probiotics and biofilms TW

Detection of biofilm producer microorganism

A- Direct observation

- Light microscope,
- Transmission electron microcroscope (TEM)
- Scanning electron microscope (SEM)
- Confocal laser scanning microscopy (CLSM)

B- Indirect observation

1- Roll plate method:

for detection of biofilm for developed on the outer surface of cylindrical materials such as catheters and vascular grafts. Material is touched and rolled on the surface of medium.

2- Congo red agar (CRA) method:

Qualitative method for detection of biofilm producer m.o, as a result of color change of colonies inoculated on CRA medium, after incubation , if black colonies with a dry crystalline consistency indicate biofilm producers, whereas colonies retained pink are non-biofilm producers.

3- Tube method (TM)

Qualitative method. Isolates are inoculated in polystyrene test tube. The occurrence of visible film lined the walls & the bottom of the tube indicate biofilm production.

4- Microtiter plate assay

Quantitative method

5- detection of biofilm-associated genes by PCR

Detection of virulence factors by amplifying target virulence genes such as biofilm genes with the usage of gene-specific primers

Probes Used In Measurment of Biofilm

Characteristic (Measured Feature)	Method (Staining probe)	Detection method
Biofilm biomass	Crystal violet assay (CV)	Visual detection, absorbance
Microbial physiological activity (viability)	Resazurin assay	Fluorescence, absorbance
	Tetrazolium salts (XTT assay, MTT assay)	Absorbance
	Fluorescein diacetate assay	Fluorescence
Biofilm matrix (EPS)	Dimethyl methylene blue assay (DMMB)	Absorbance

Measurment of Biofilm Cont...

Multimode plate reader (spectrophotometry, flourescence and luminescence)

Measurment of Biofilm Cont...

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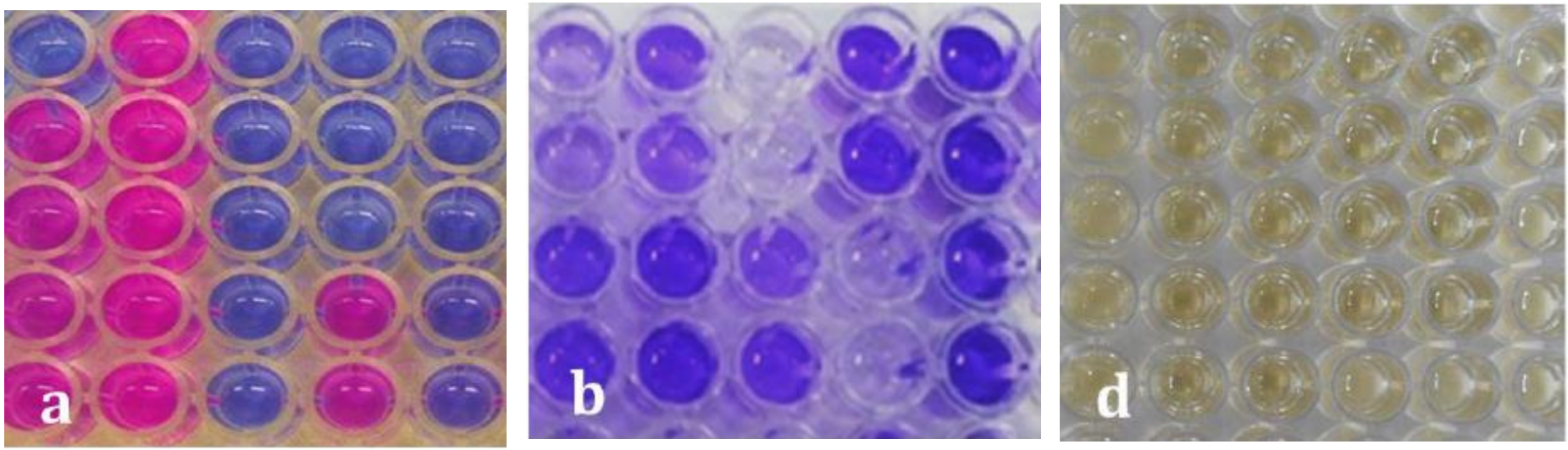

(a) Resazurin assay, (b) crystal violet staining assay, (d) turbidity measurements

Antifungal therapy of Candida biofilms: Past, present and future

Past

1-Polyenes (Amphotericin B): 1950s

- It is broad spectrum fungicidal agent. It was used for invasive fungal infections including candidiasis.
- These amphipathic compounds act as a sponge extracting ergosterol from fungal cell membrane → pores → leakage of cellular components → death of fungal cells.
- Nephrotoxic.
- It is less potent 10 times in biofilm than planktonic cells and it reached toxicity.
- Liposomal formulation of Amp B was active against *Candida* biofilm through better penetration across biofilm.

2- Azoles (Fluconazole): 1980s-1990s

- It was 1st line therapy against *candida* infections, fungistatic but more safe antifungal agent than amphotericin B.
- It also targets ergosterol but by inhibiting its biosynthetic pathway (cytochrome P-450).
- The resistance of planktonic *Candida* was developed against fluconazole.
- MIC of fluconazole against biofilm was 1000 times >planktonic candida.

3- Echinocandins (capsofungin) 1970s-2000s

- It is semisynthetic lipopeptide antibiotic that inhibit 1,3 β-D-glucan synthase (key enzyme for the glucan synthesis that is the main structure of candida cell wall)
- It has potent fungicidal activity with excellent safety profile and active against *Candida* biofilm.
- Emergence of resistance through mutation in the gene encoding target enzyme.

Present

1- Rezafungin: (long acting echinocandin)

- It has activity against biofilm formation and mature biofilm.

2- Ibrexafungerp: (New semi-synthetic terpenoid)

- It targets 1,3- β-D- glucan synthase but different from echinocandins.
- It is active against *Candida* spp. biofilm.

3-Fosmanogepix & Manogepix:

- They are broad spectrum antifungal agents. They inhibit *C. albicans* adherence and biofilm formation

Future

1- Screening of chemical libraries to identify new compounds with inhibitory activity against *Candida* biofilm

- A complementary approach for screening in the chemical libraries for compounds that synergize with or potentiate the anti-Candida biofilm of current clinically- used antifungal agents.

2- Turbinmicin:

- It is produced by the associated microbiome of a marine animal that inhibit the vesicle-delivered biofilm matrix.

3- EntV (bacteriocin produced by *E. faecalis*)

- Antivrulence of can inhibit *C. albicans* morphogenesis, biofilm formation *Enterococcus faecalis*, and overall virulence (*C. albicans* filamentation and biofilm formation both in-vitro and in-vivo.

4- Repurposing:

- Repositioning of new therapeutic indications for already existing drugs which is faster, cheaper, more successful than “de novo” drug discovery
- Examples: uranofin, ebselen, alexidine & niclosamide

5- Nanotechnological approaches: (Nanomaterials, Nonoparticles or Nanoantibiotics)

- size < 100 nm.
- In nanometric scale, they display new or improved physicochemical properties>
- Metal nanoparticles e.g. Silver nanoparticles (Ag NPs); Polymeric nanoparticles (chitosan, curcumin).
- Limitation of use of nanoparticles in systemic therapy.

6- Other approaches

- Antimicrobial peptides
- probiotics
- modulator of quorum sensing
- Photodynamic therapy (PDT): action of reactive oxygen species generated by the photoactivation of a photosensitizer by a light source.
- Novel surface coatings developments that inhibit *Candida* attachment &/or subsequent biofilm formation

Conclusion

- The lack of effective antifungal therapeutics contributes to the excess morbidity and mortality rates associated with biofilm-associated candidiasis.
- Lessons from the past and our increasing understanding of Candida biofilms offer new opportunities for the development of novel therapeutics to combat the threat that these devasting infections pose to an increasing number of at-risk patients.

Thank you