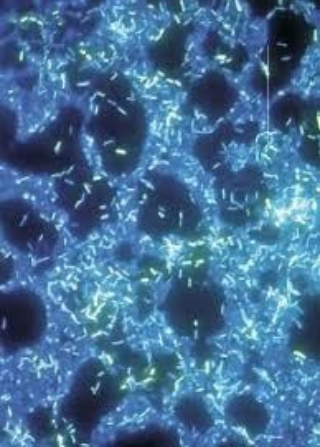
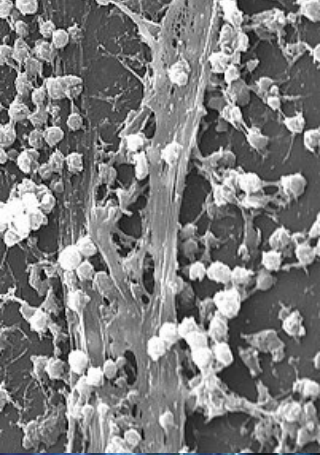


MicroBioma humano e Biofilmes

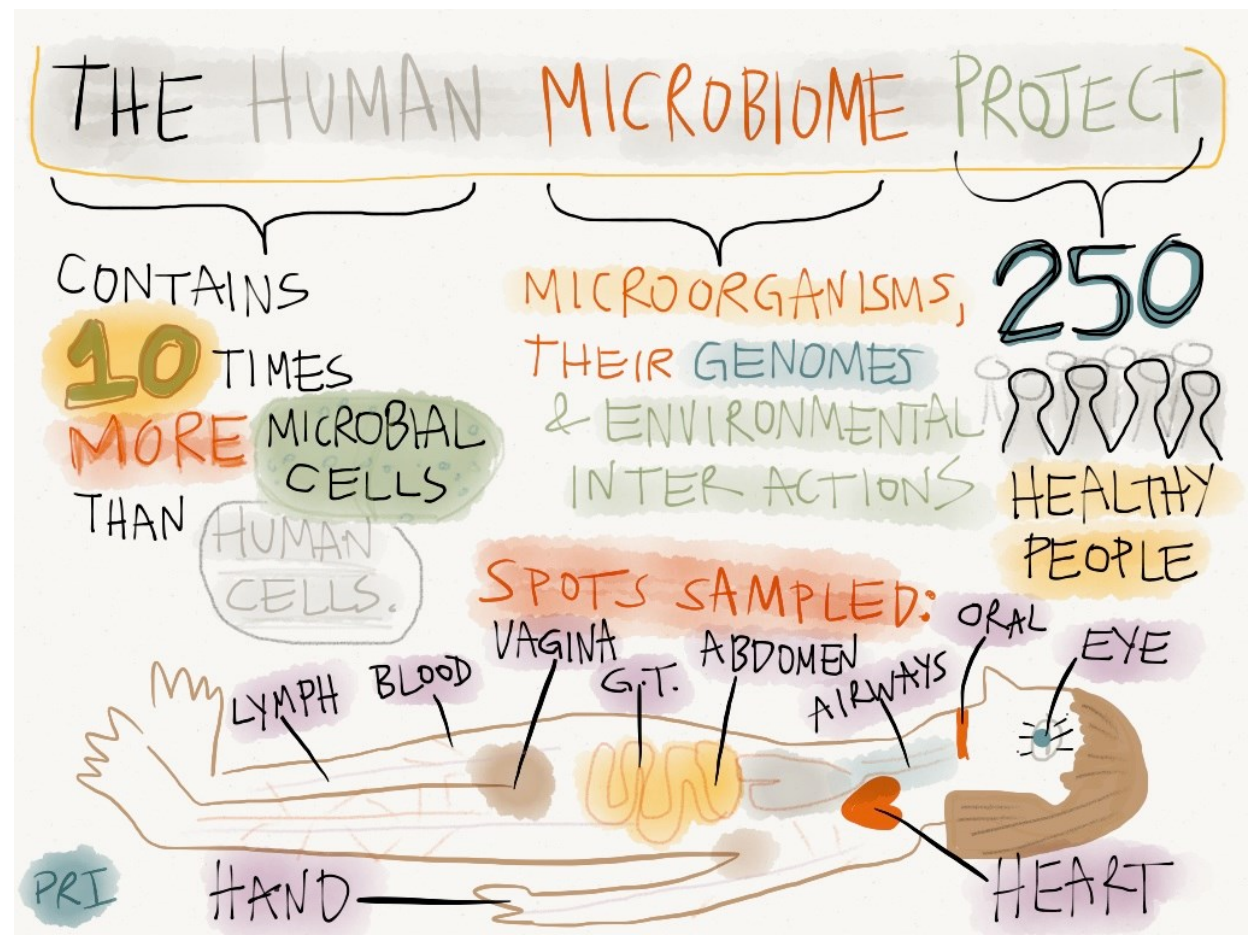
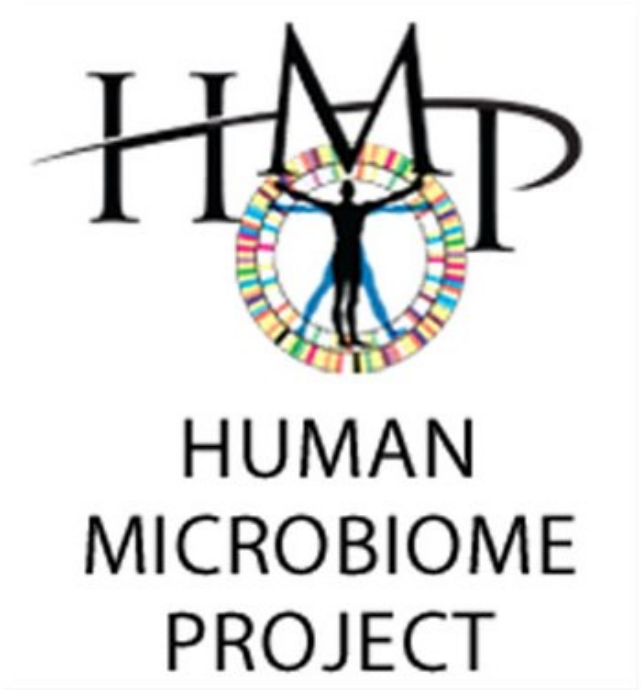
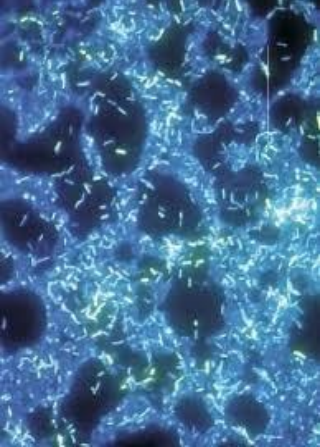
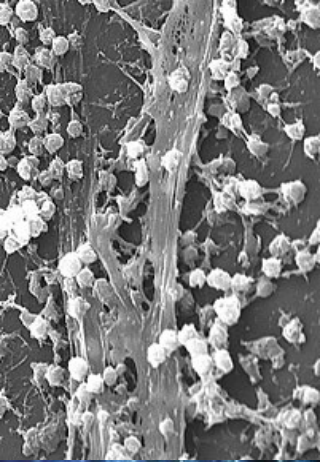
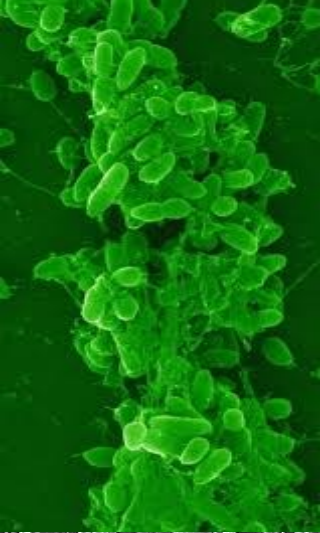
Fisiologia Celular e Molecular

Juliana Melo



O que é microbioma ?

MicroBioma Humano



MicroBioma Humano



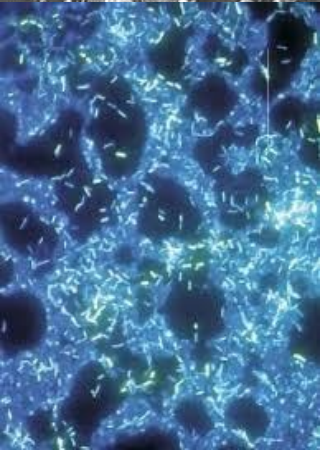
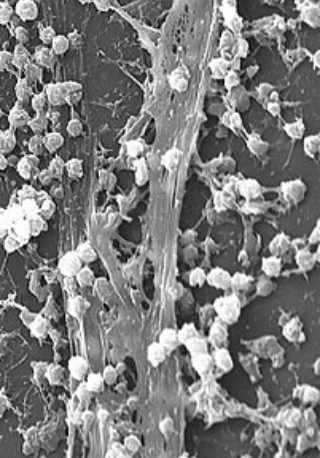
THE RESEEARCHERS FOUND

1,000 STRAINS OF BACTERIA ON EACH PERSON!

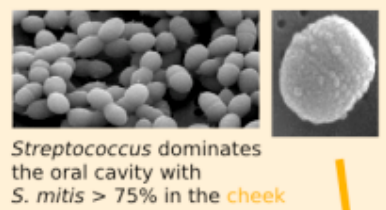


THE MICROBIOME STARTS AT BIRTH. AS BABIES PASS THROUGH THE BIRTH CANAL, THEY PICK UP THE BACTERIA FROM THE MOTHER'S VAGINAL MICROBIOME.

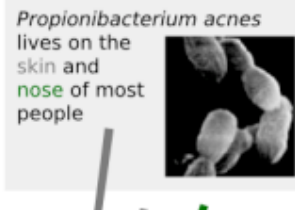
PRI



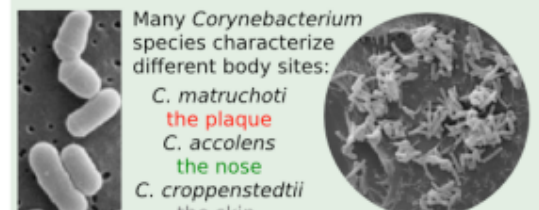
A map of diversity in the human microbiome



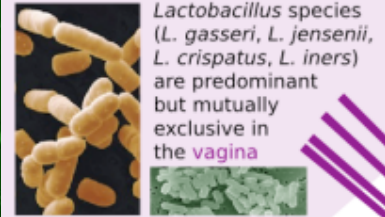
Streptococcus dominates the oral cavity with *S. mitis* > 75% in the **cheek**



Propionibacterium acnes lives on the skin and **nose** of most people



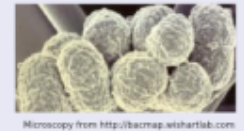
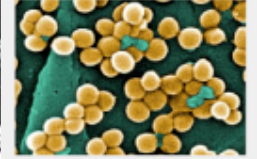
Many *Corynebacterium* species characterize different body sites:
C. matruchoti the **plaque**
C. accolens the **nose**
C. croppenstedtii the **skin**



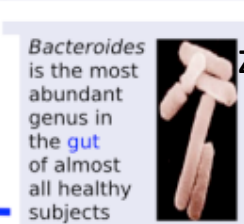
Lactobacillus species (*L. gasseri*, *L. jensenii*, *L. crispatus*, *L. iners*) are predominant but mutually exclusive in the **vagina**



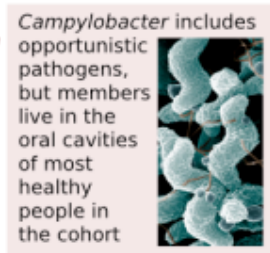
Staphylococcus epidermidis colonizes external body sites



Several *Prevotella* species are present in the gastrointestinal tract. *P. copri* is present in 19% of the subjects and dominates the **intestinal** flora when present



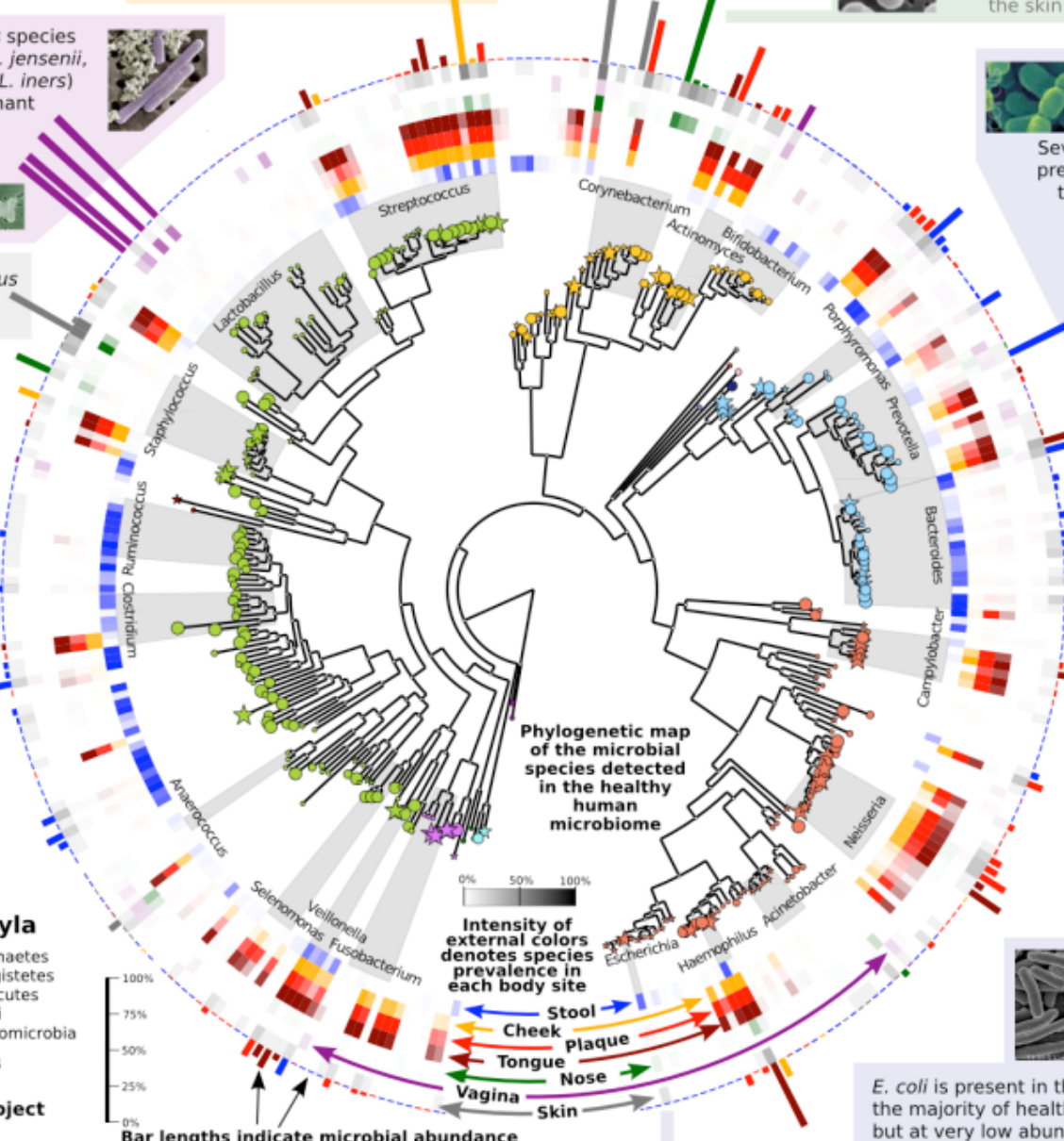
Bacteroides is the most abundant genus in the **gut** of almost all healthy subjects



Campylobacter includes opportunistic pathogens, but members live in the oral cavities of most healthy people in the cohort



E. coli is present in the **gut** of the majority of healthy subjects but at very low abundance



○ Commensal microbes
 ☆ Potential pathogens

The four most abundant phyla

- Actinobacteria
- Bacteroidetes
- Firmicutes
- Proteobacteria

Low abundance phyla

- Chloroflexi
- Cyanobacteria
- Euryarchaeota
- Fusobacteria
- Lentisphaerae
- Spirochaetes
- Synergistetes
- Tenericutes
- Thermi
- Verrucomicrobia

National Institutes of Health
 Human Microbiome Project

N. Segata & C. Huttenhower
<http://huttenhower.sph.harvard.edu>
generated using Cytoscape and rCytoscape (data from Human Microbiome Project)

Bar lengths indicate microbial abundance (colored by body site of greatest prevalence)

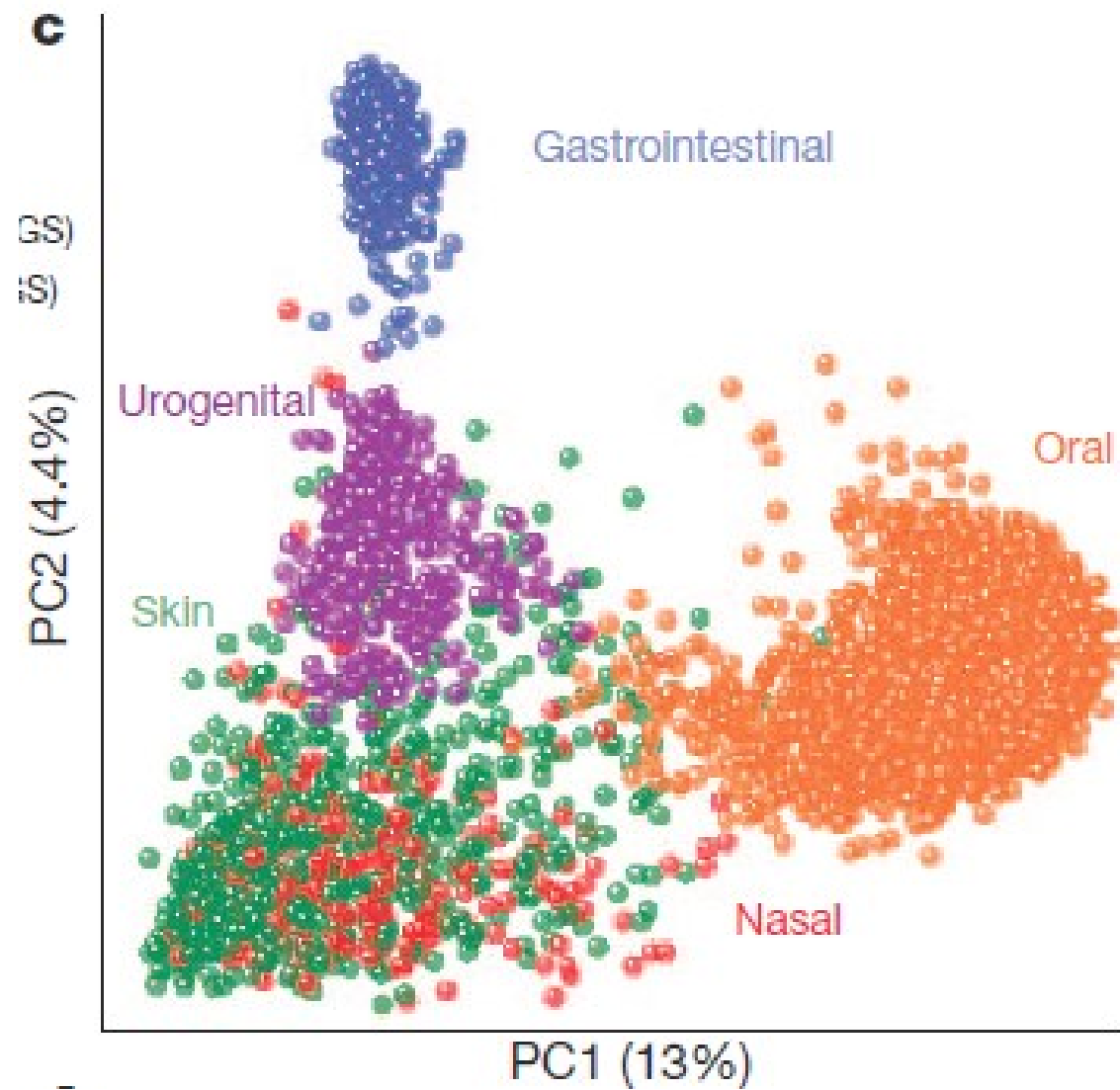
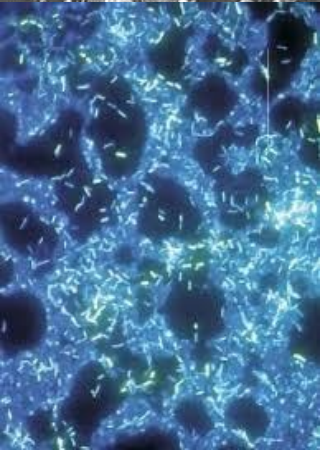
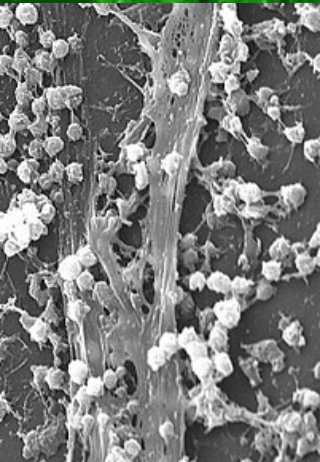
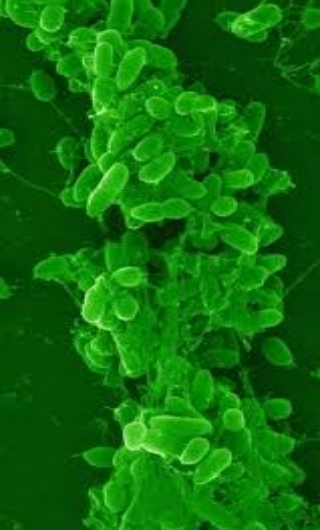
Phylogenetic map of the microbial species detected in the healthy human microbiome

Intensity of external colors denotes species prevalence in each body site

0% 50% 100%
 Stool
 Cheek
 Plaque
 Tongue
 Vagina
 Skin

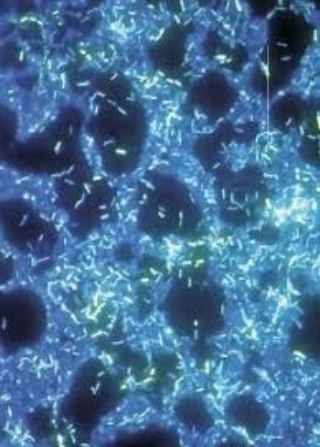
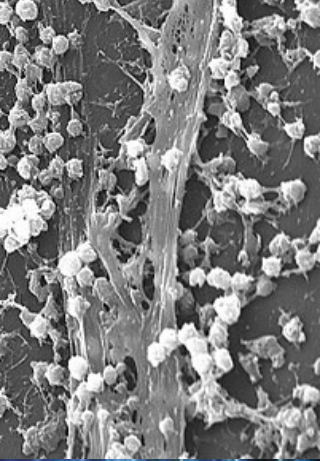
Mapa representativo da caracterização do microbioma nas várias zonas do corpo humano e a sua abundância.

MicroBioma Humano

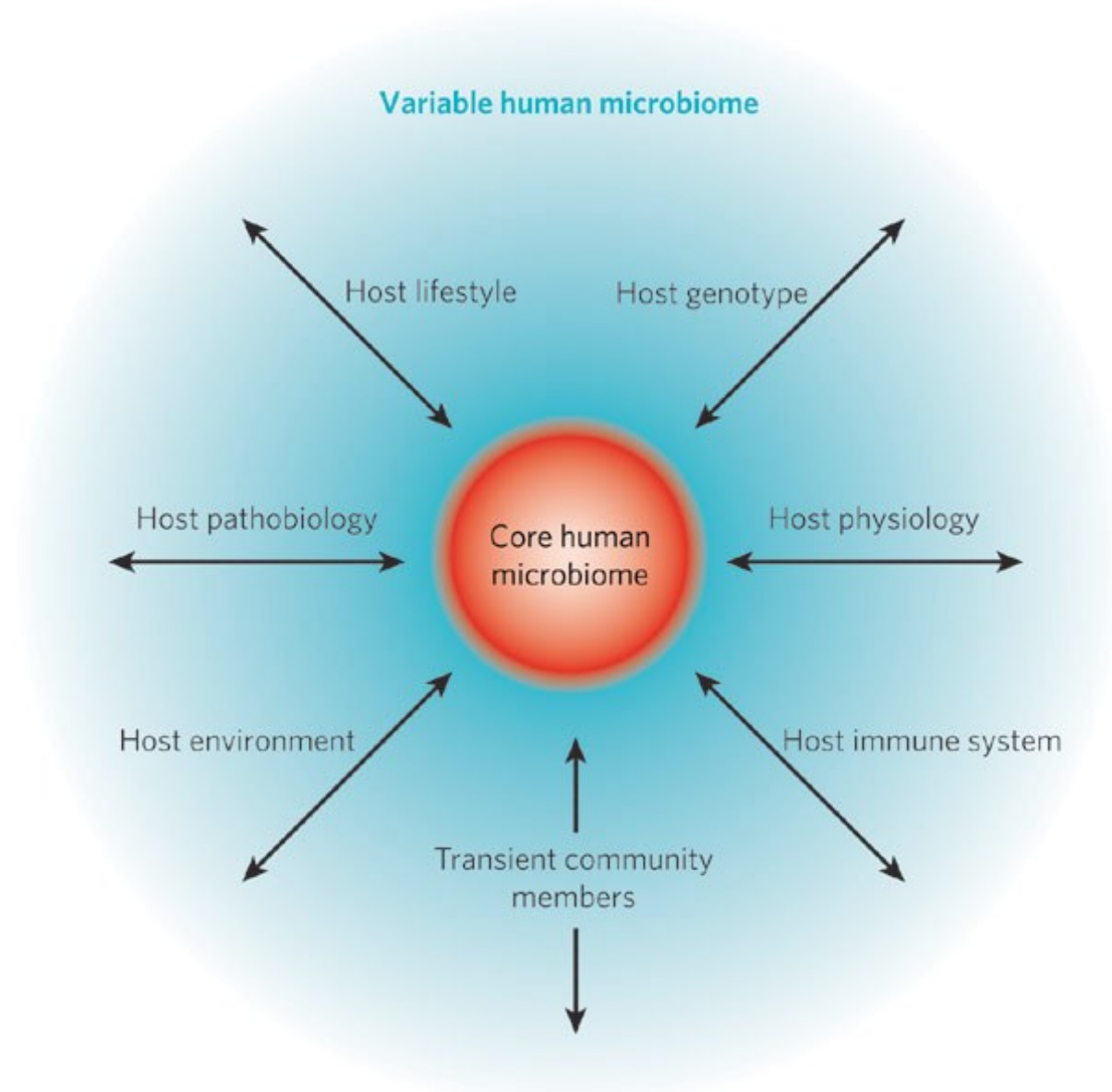
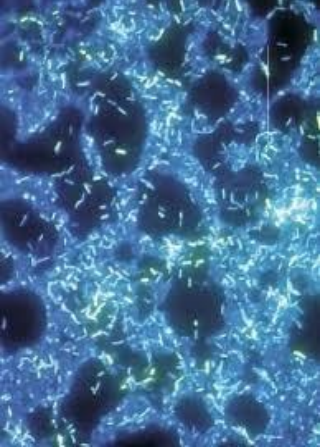
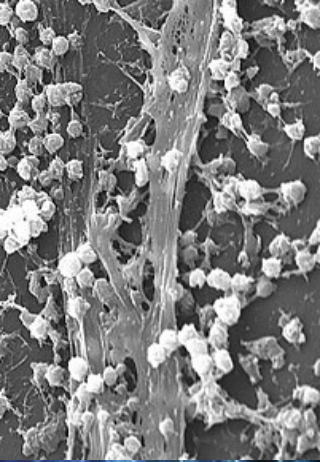


MicroBioma Humano

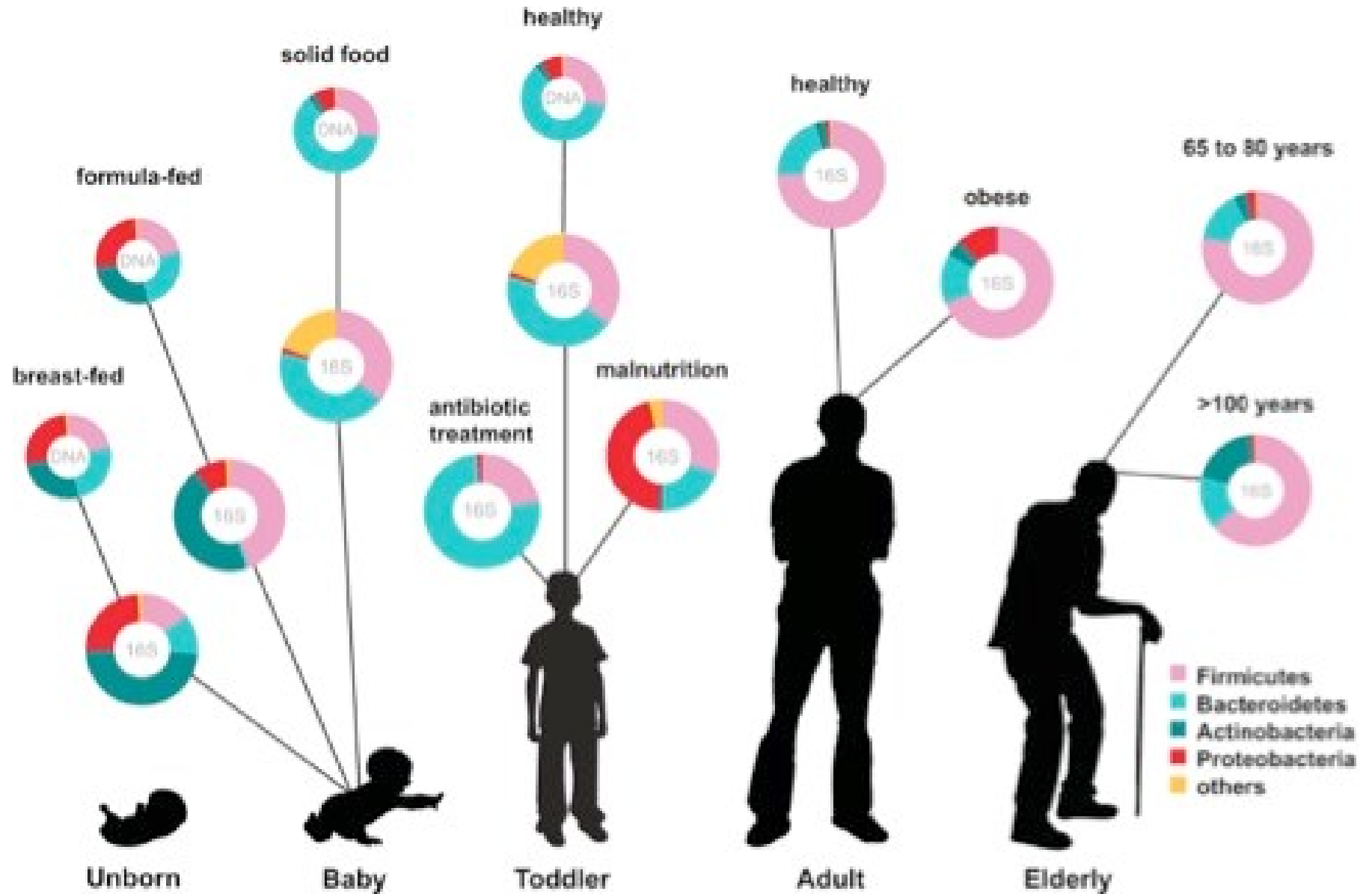
O que pode influenciar o microbioma?



MicroBioma Humano

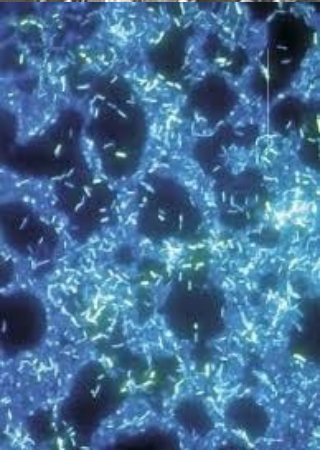
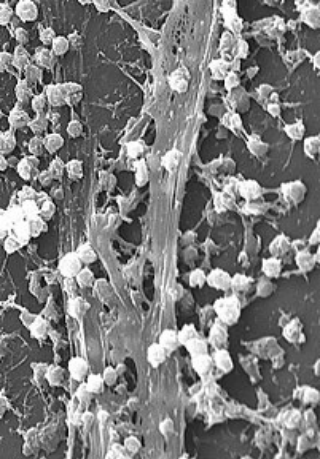


MicroBioma Humano



Biofilms

Os microrganismos presentes no
nosso corpo são capazes de
formar biofilmes?



Biofilms

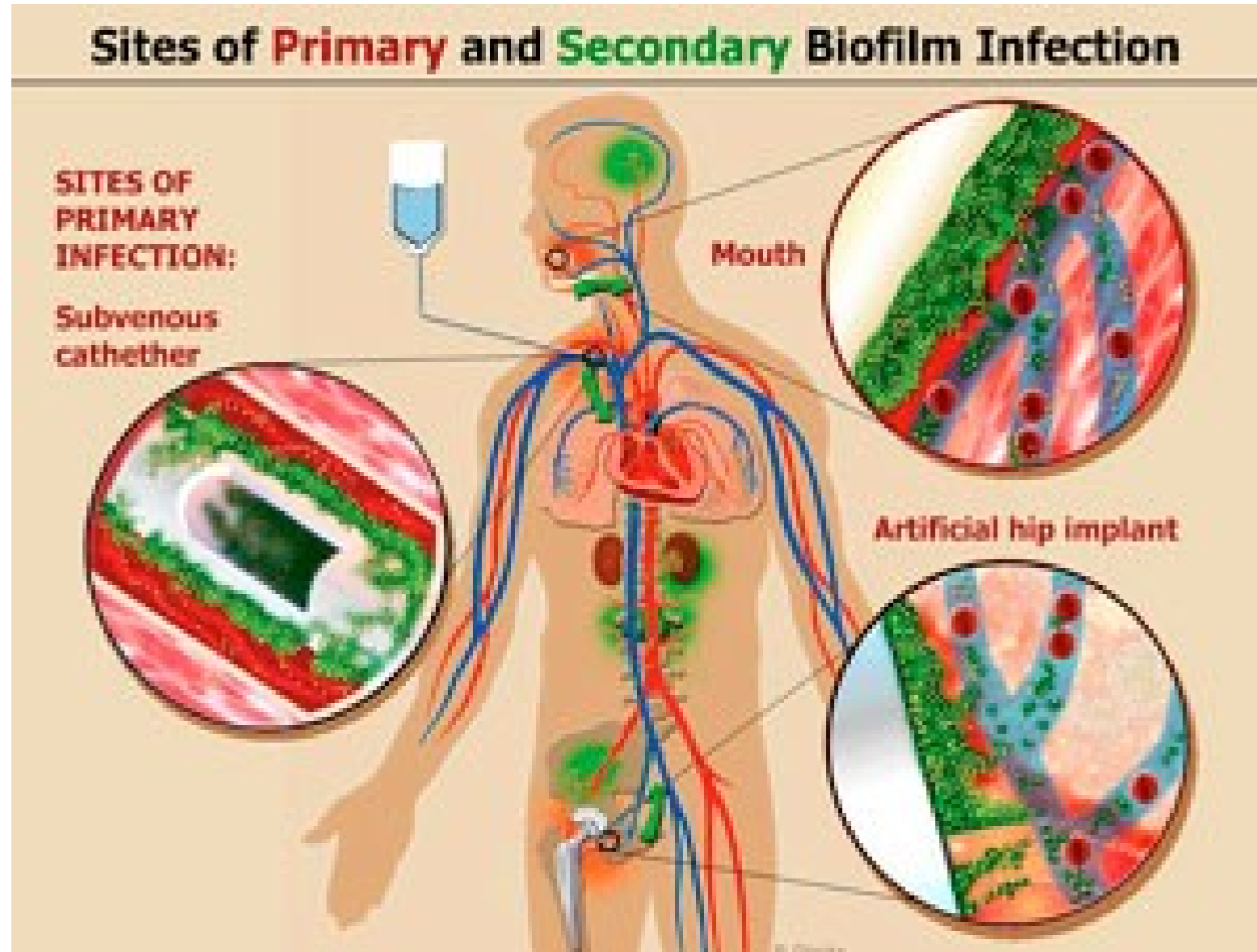
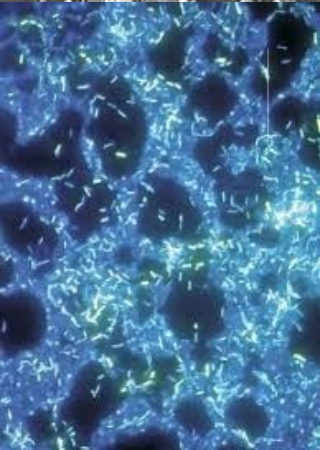
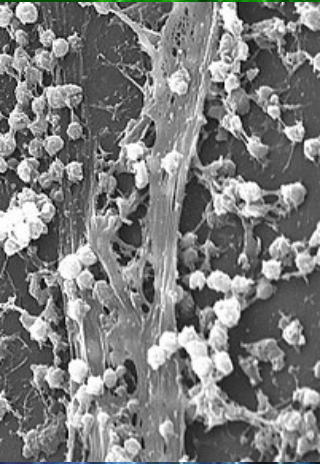
Sites of **Primary** and **Secondary** Biofilm Infection

**SITES OF
PRIMARY
INFECTION:**

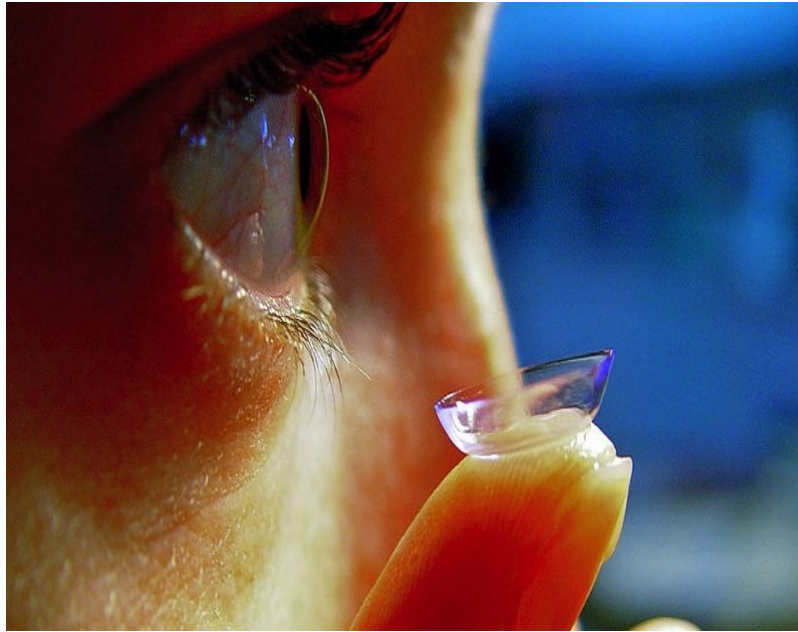
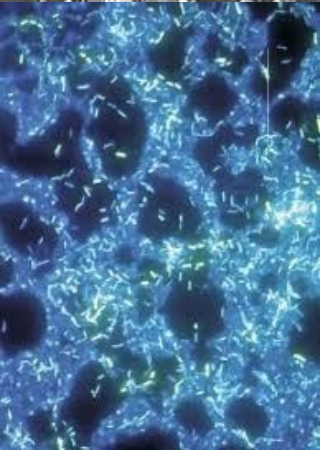
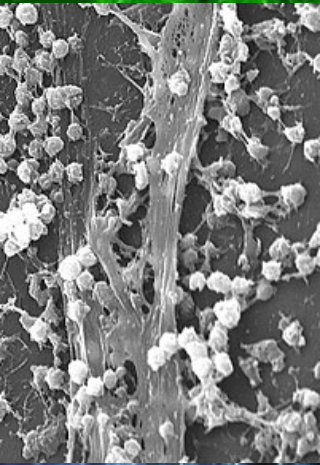
**Subvenous
catheter**

Mouth

Artificial hip implant



Biofilms



Early observations: body teeming with microbial life



+



=



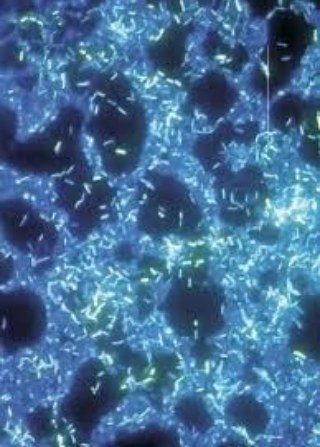
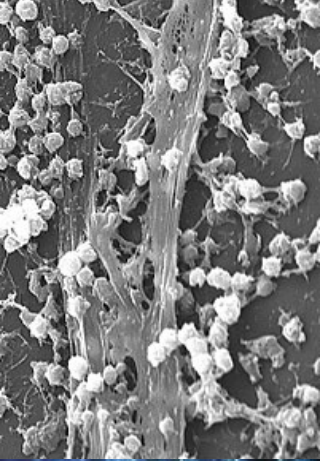
“...animalcules were in such enormous numbers, that all the water...seemed to be alive.” — van Leeuwenhoek (1683)

Biofilms

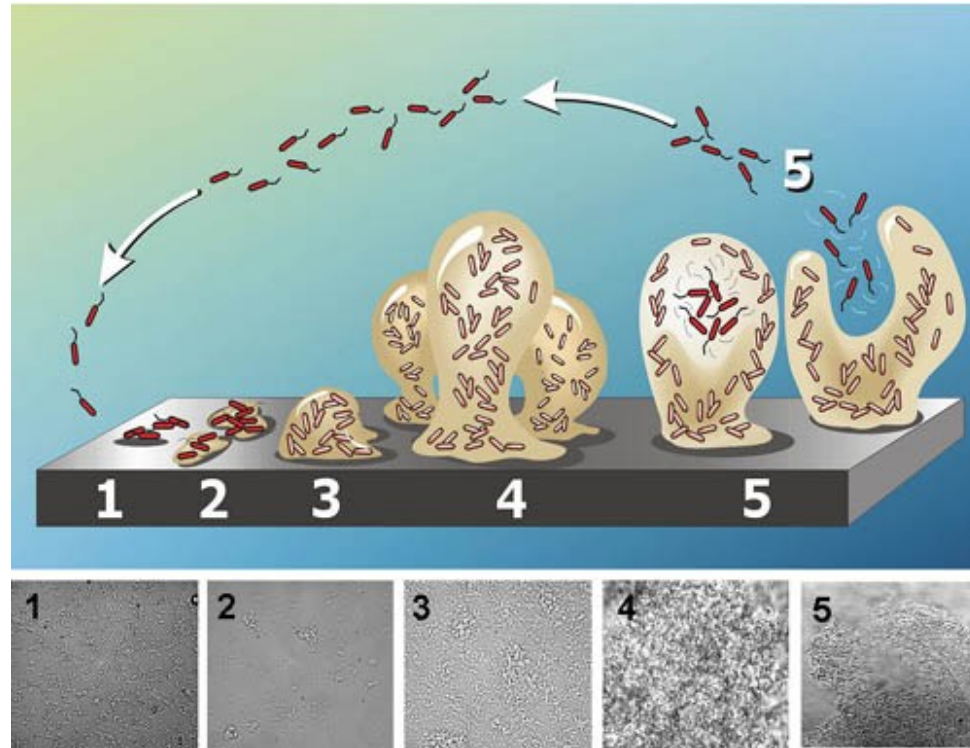
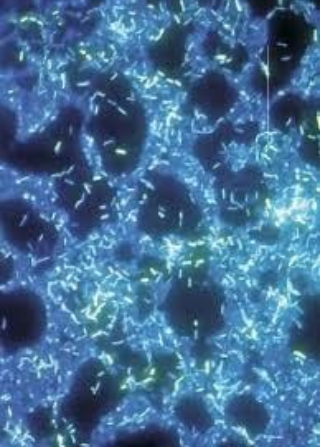
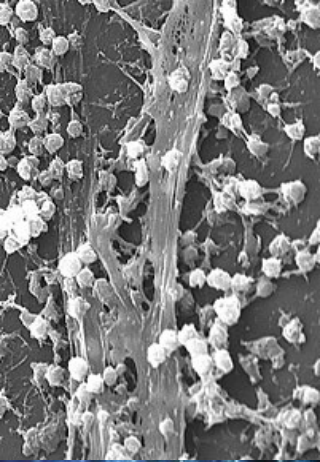
Definition of a Biofilm:

Biofilms are communities of microorganisms in a matrix that joins them together and to living or inert substrates

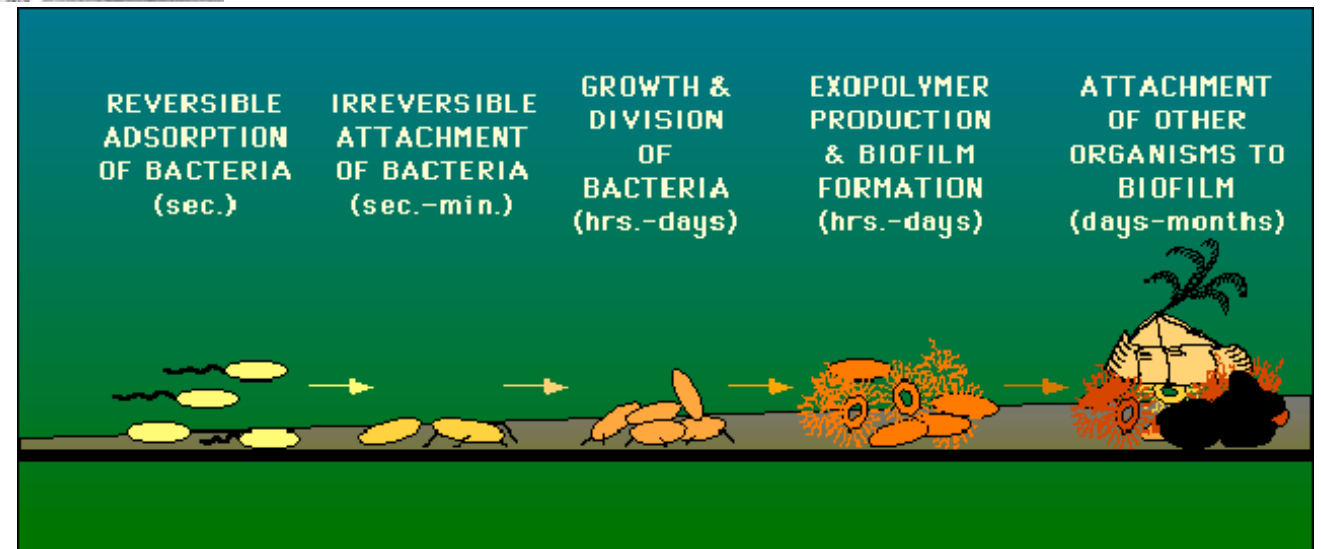
Biofilms are surface-attached communities of bacteria, encased in an extracellular matrix of secreted proteins, carbohydrates, and/or DNA, that assume phenotypes distinct from those of planktonic cells



Biofilm formation stage

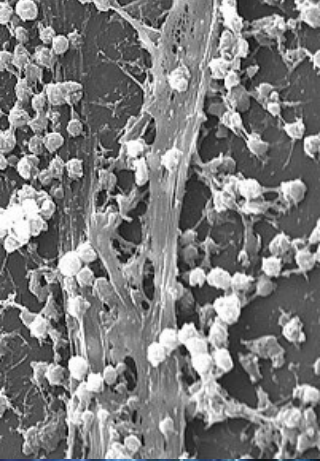


Aproximação da superfície
Acaso?





Pathogens that have been studied for the formation of biofilms



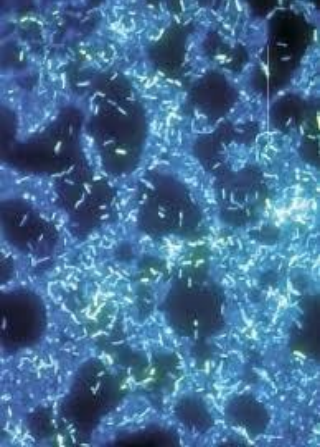
Staphylococcus aureus

Staphylococcus mutans

Salmonella typhi

Enterococcus faecalis

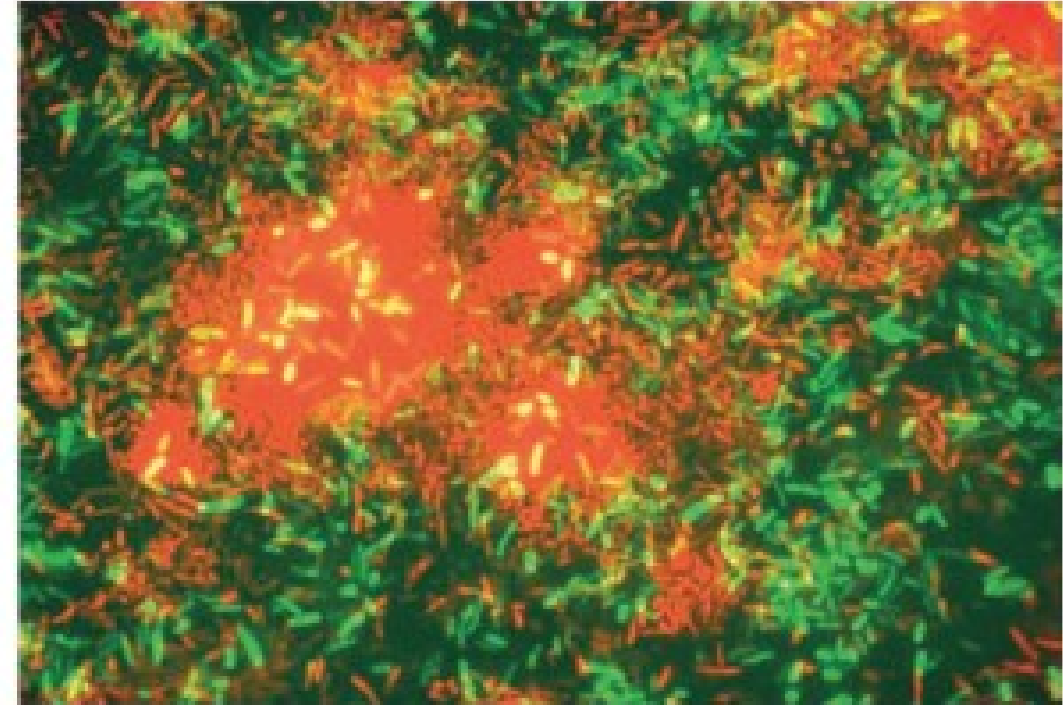
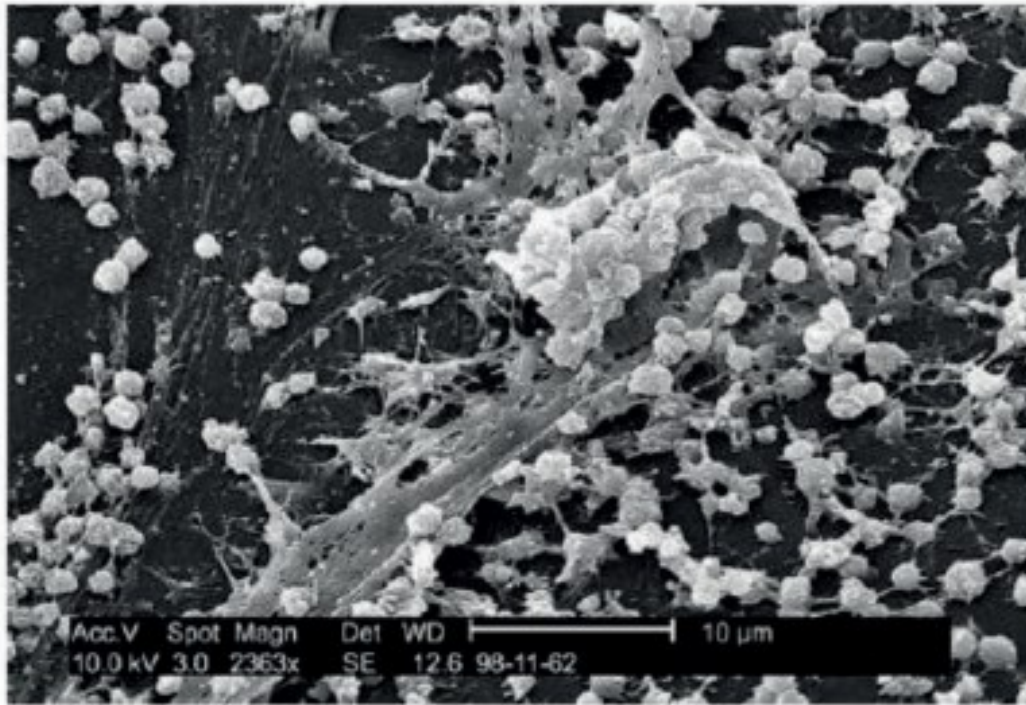
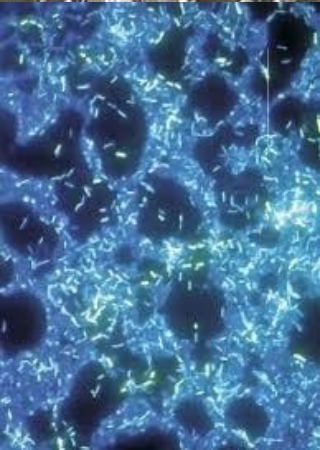
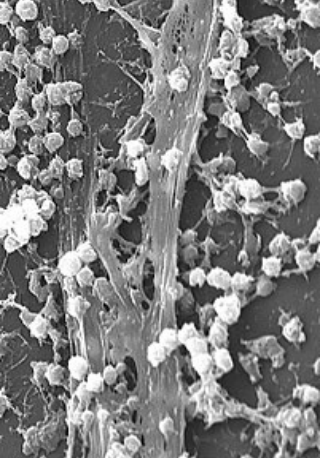
Pseudomonas aeruginosa



Detection of biofilm producer microorganism

Direct observation:

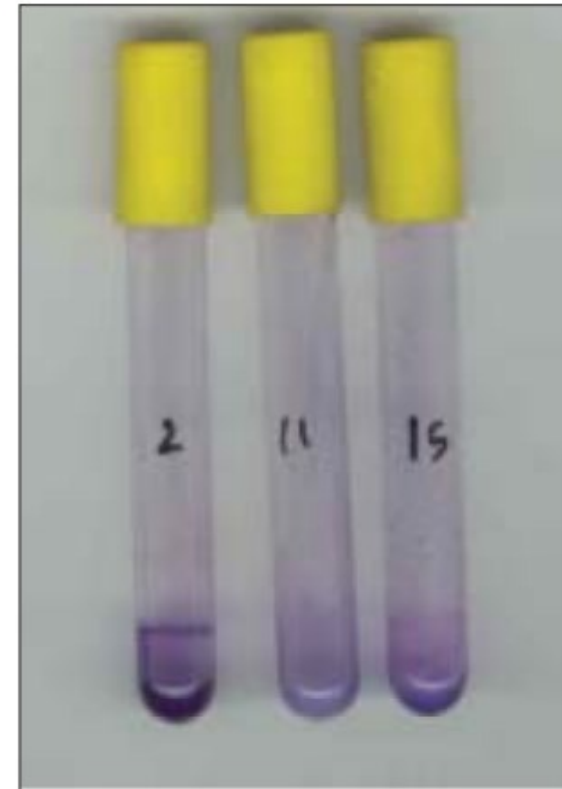
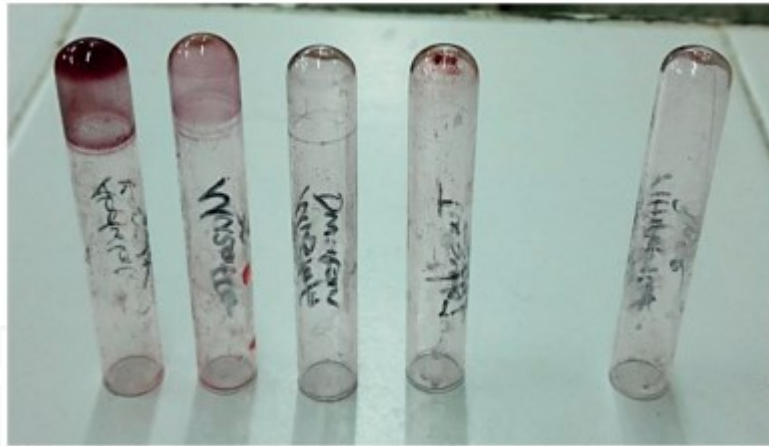
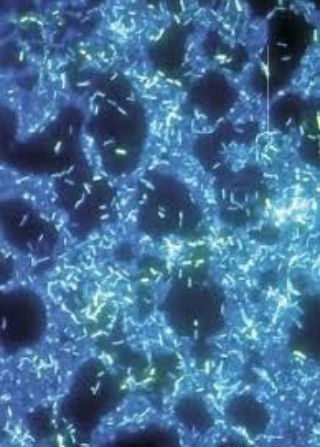
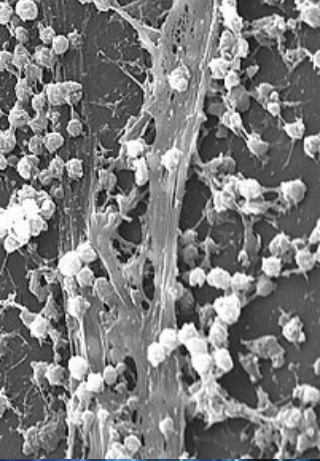
Microscope



Detection of biofilm producer microorganism

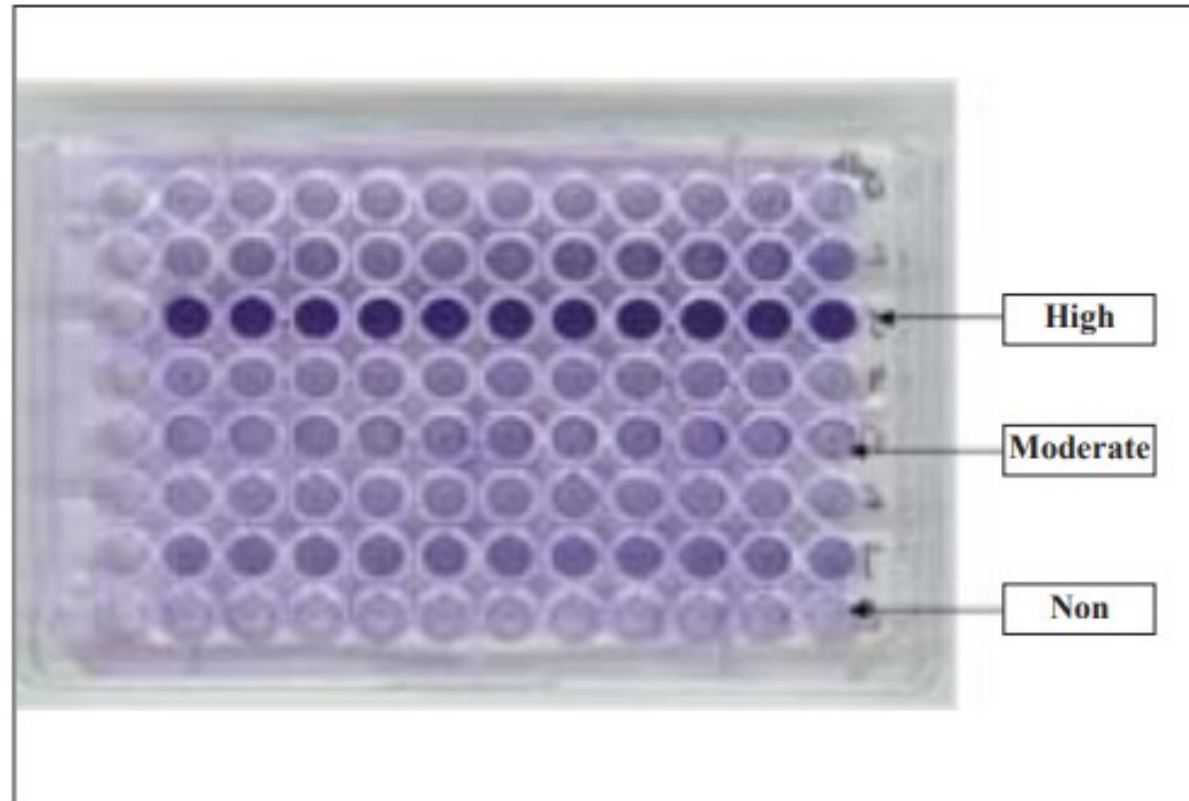
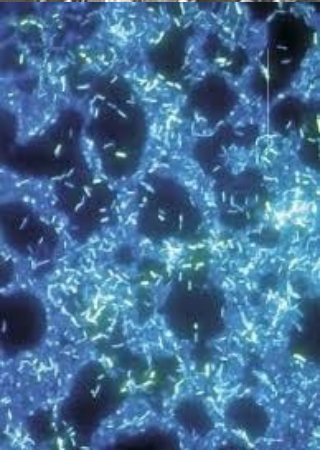
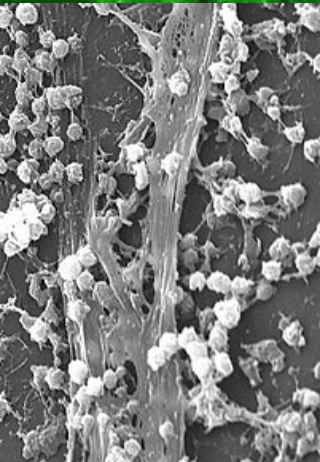
Indirect observation:

Tube method



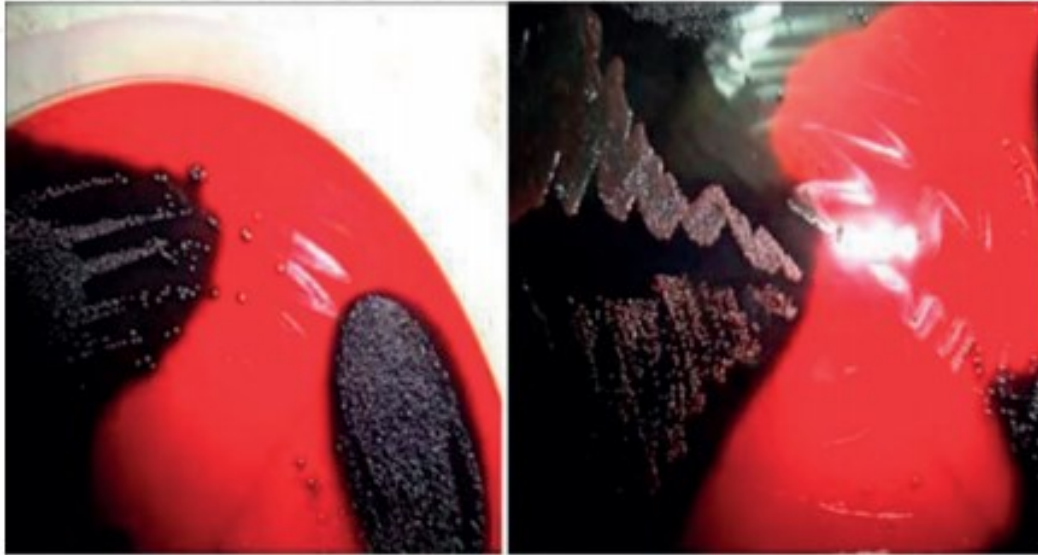
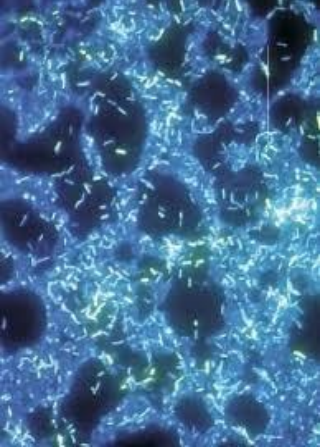
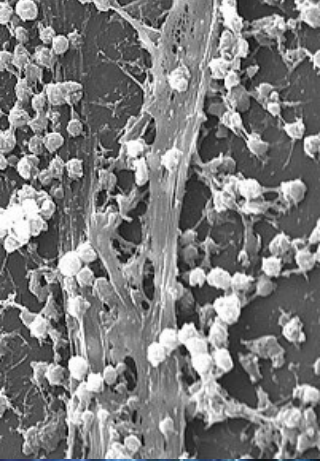
Detection of biofilm producer microorganism

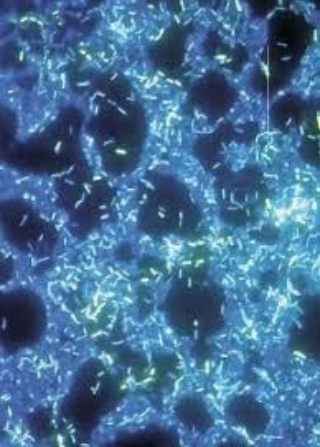
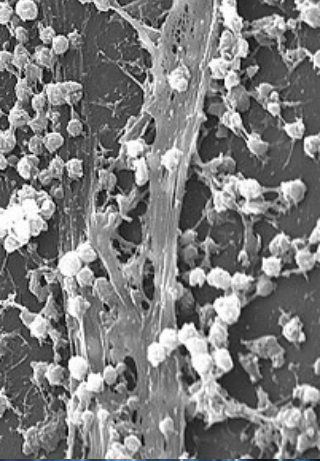
Detection of biofilm production by microtiter plate assay Crystal Violet



Detection of biofilm producer microorganism

Congo red agar method

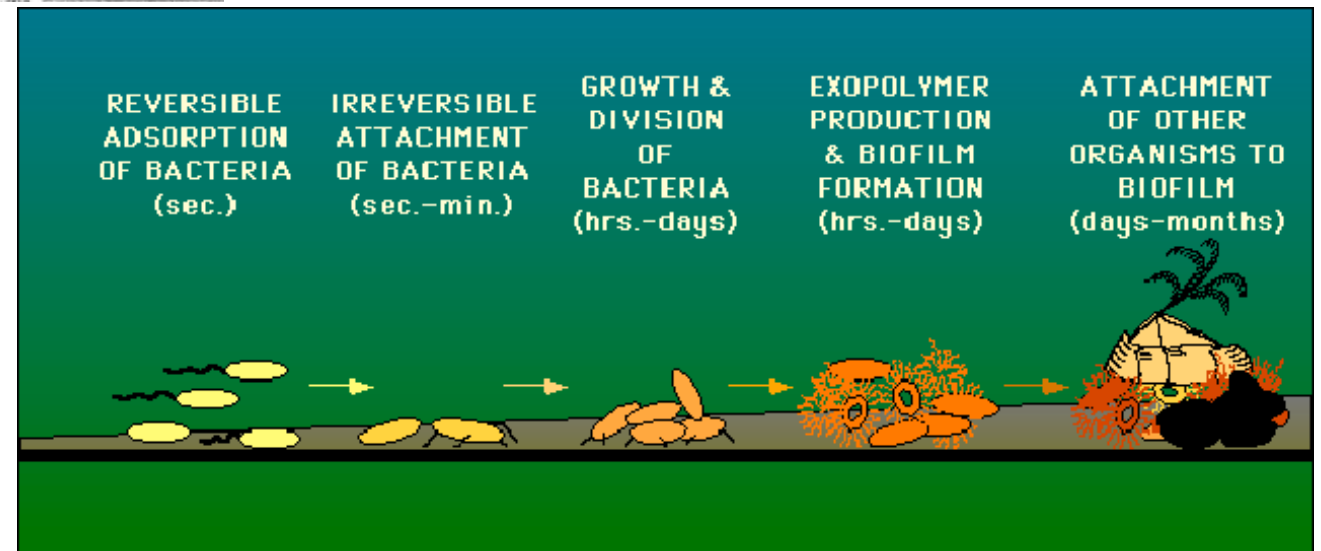
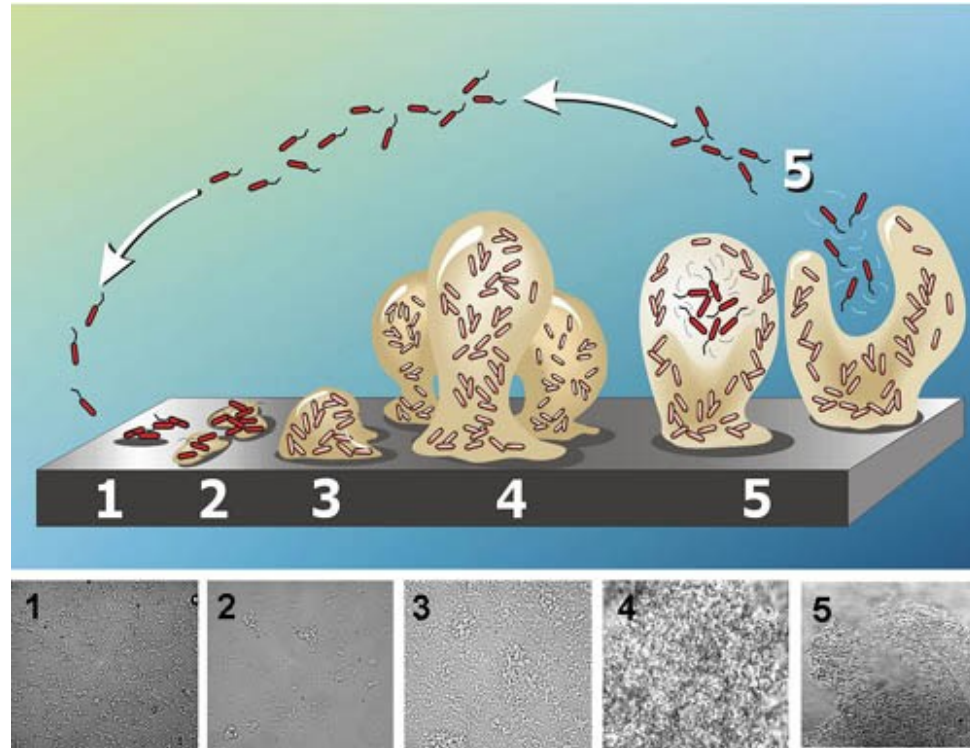
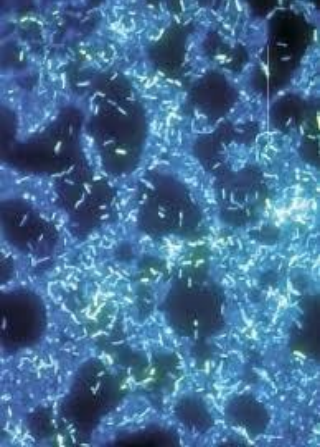
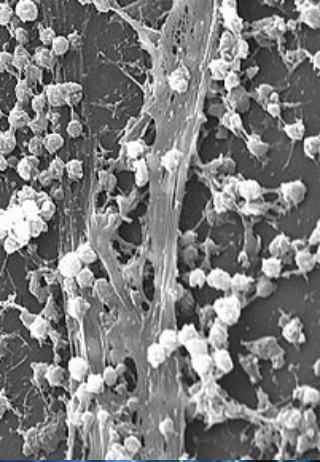


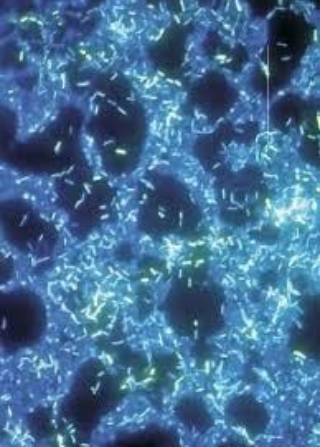
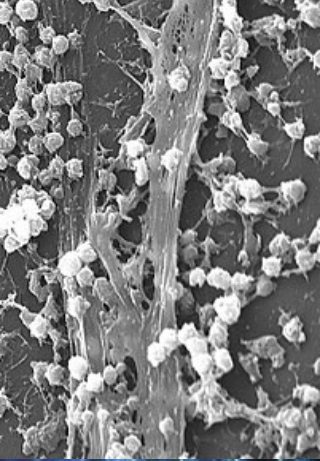


Practical work

Resistance to antibiotics

Biofilm formation stage

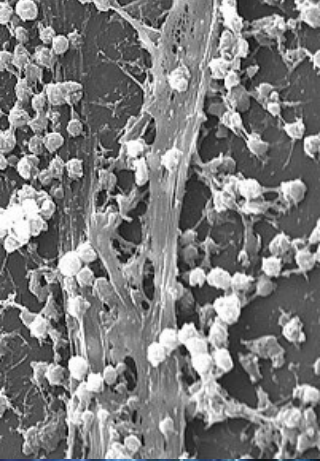




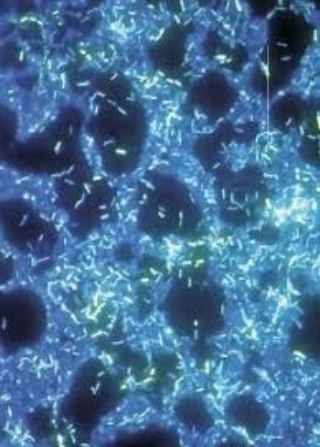
Objetivo: detetar diferenças na resistência aos antibióticos em células planctónicas e formadoras de biofilmes de *E. coli* e *B. subtilis*



Escherichia coli (Gram negativa) é uma das espécies mais abundantes entre as bactérias anaeróbicas facultativas que se encontram presentes no trato intestinal.



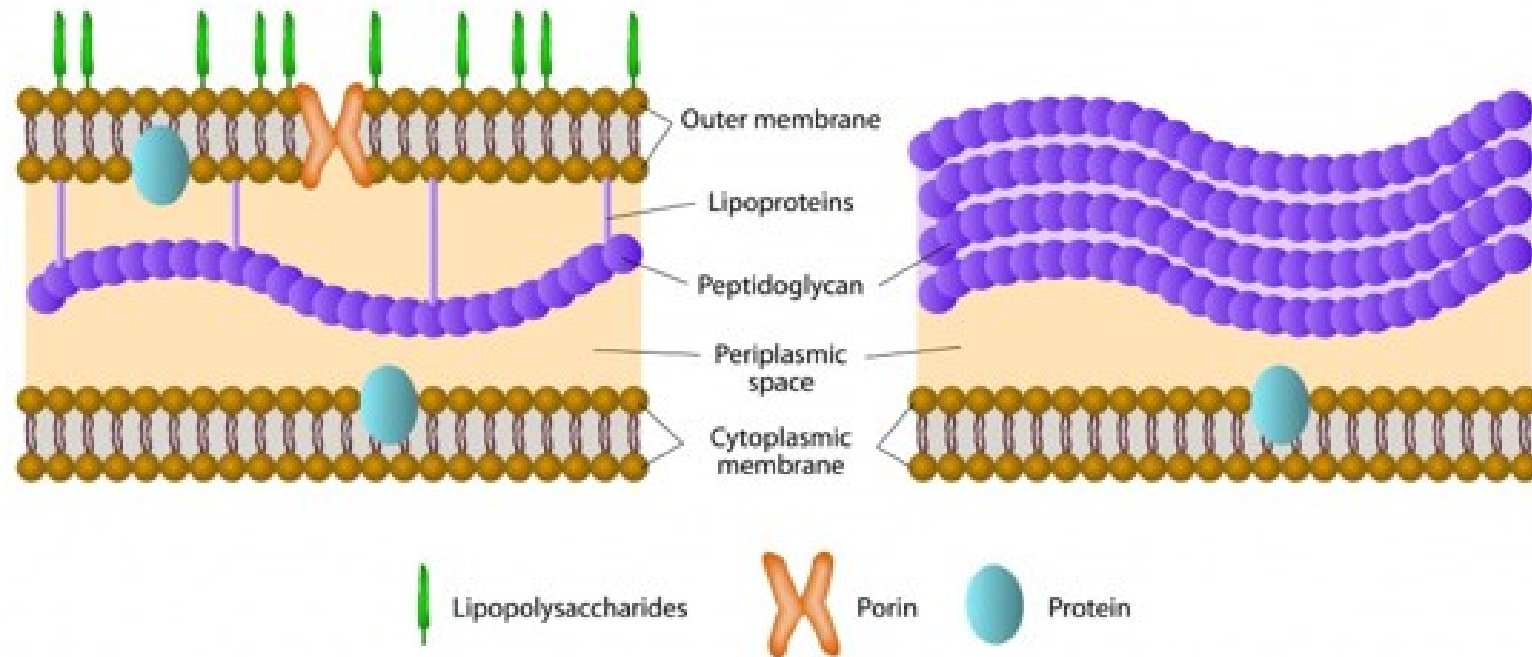
Bacillus subtilis (Gram positiva) embora exista como comensal na flora intestinal humana é mais característico do solo e das águas, sendo também conhecido pela sua capacidade de formar biofilmes.



Estrutura da membrana

GRAM-NEGATIVE

GRAM-POSITIVE



As bactérias Gram-negativas possuem uma camada mais fina de peptidoglicanos, além de outra camada composta por lipídios e proteínas.

As bactérias Gram-positivas possuem apenas uma camada de peptidoglicanos, que nesse caso é mais espessa.



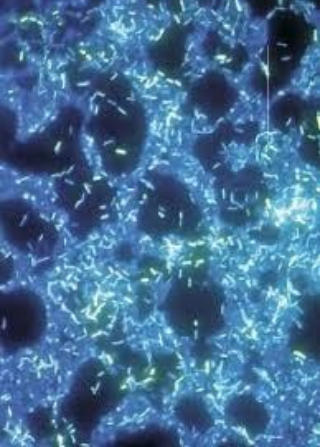
Antibióticos utilizados e atuação

O cloranfenicol liga-se à subunidade 30S do ribossomo pela inibição da peptidil transferase, responsável pela extensão da cadeia peptídica.



A rifampicina é um inibidor da RNA polimerase,

Canamicina: inibe a síntese de proteínas mediante união irreversível às proteínas ribossômicas 30S.

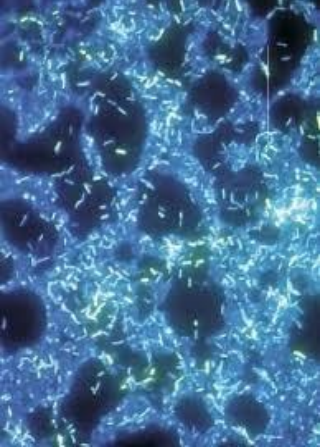
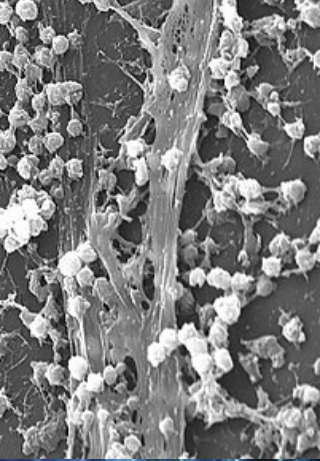


Ampicilina: Beta-lactanico atua a nível da síntese da parede celular.



Sésseis (Biofilmes) vs planctónicos

Qual é a melhor estratégia de sobrevivência das bactérias?



Material utilizado

Cultura crescida em caixa de Petri ou overnight

50 mL de soro fisiológico 0,85% - Como se faz e porque utilizamos?

Zaragatoa;

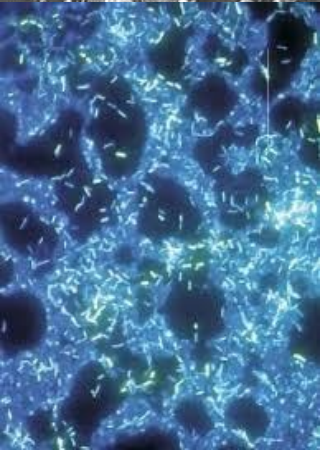
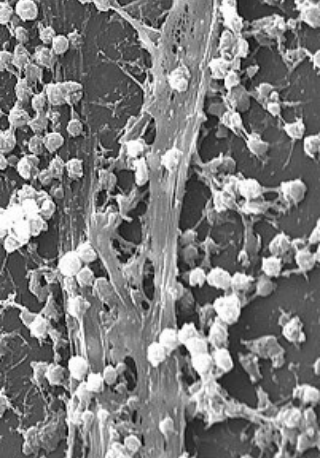
Escala de McFarland;

Meio de cultura NB;

Microplaca;

Violeta de Cristal;

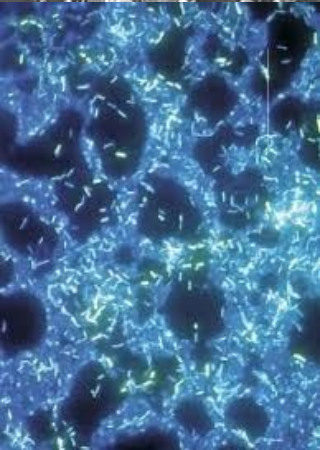
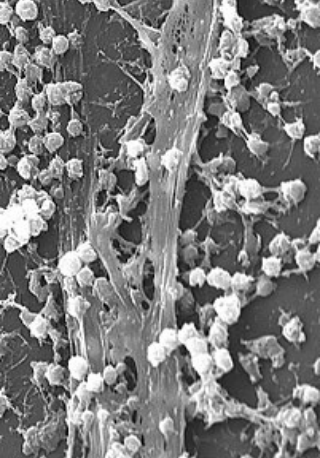
Antibióticos.



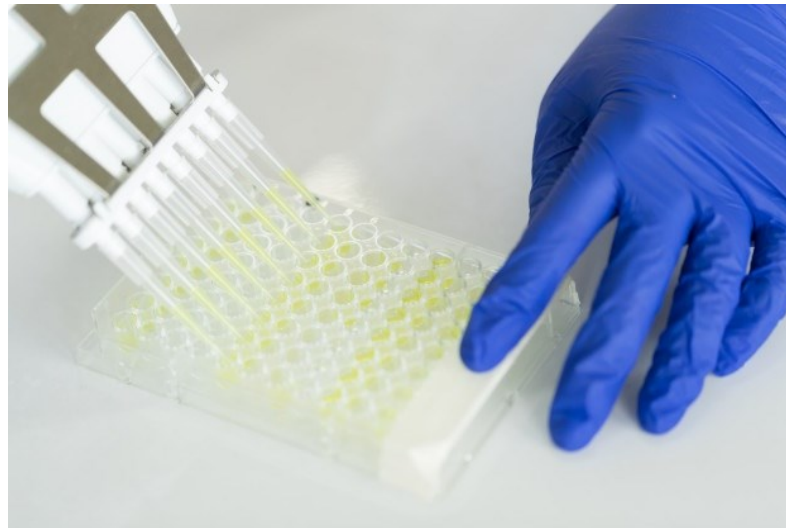
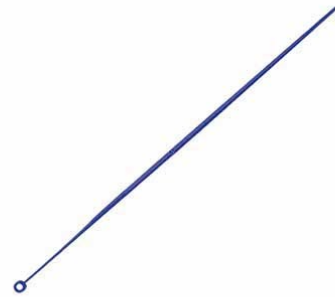
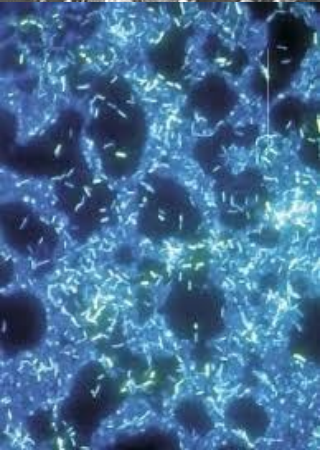
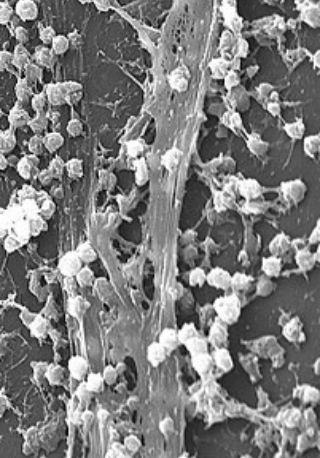
Preparação do soro fisiológico e corante

Cálculos para preparação do soro fisiológico 0,85% para um volume de 50 mL.

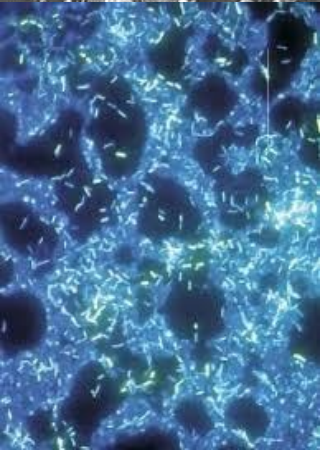
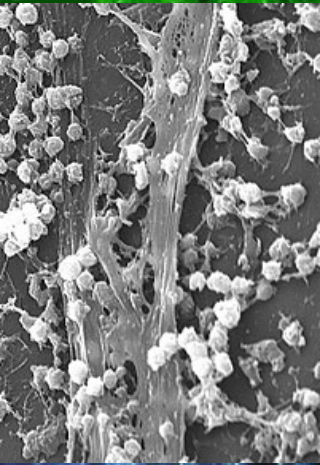
Solução de violeta de cristal 0,1% para um volume de 50 mL.



Procedimento



Procedimento:



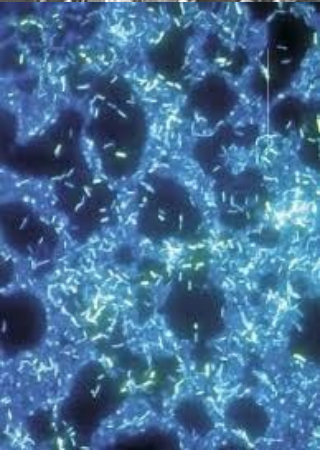
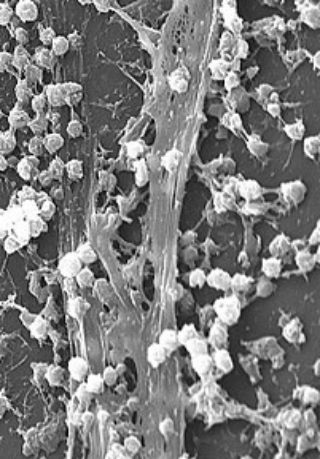
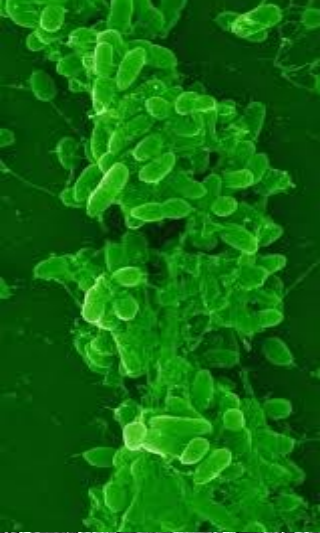
24 horas de incubação apos a adição de antibióticos

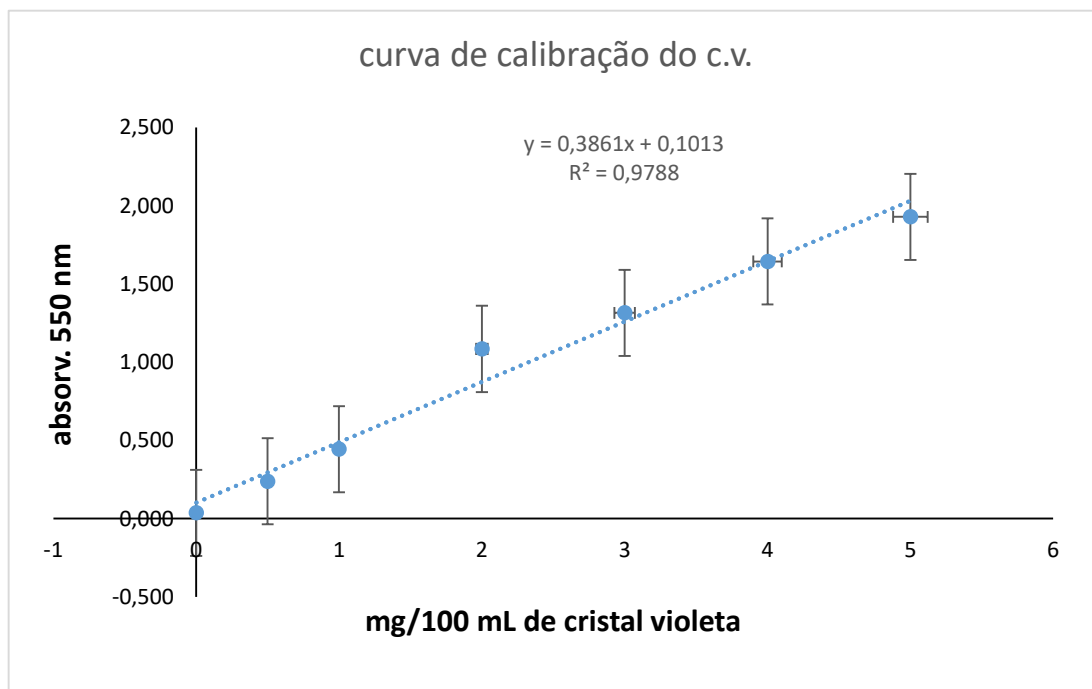
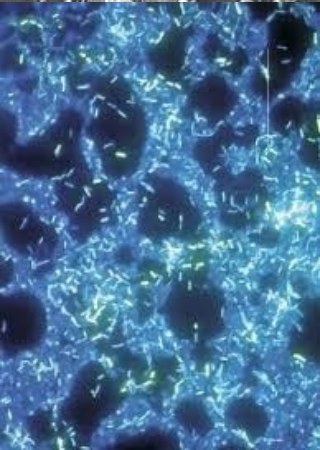
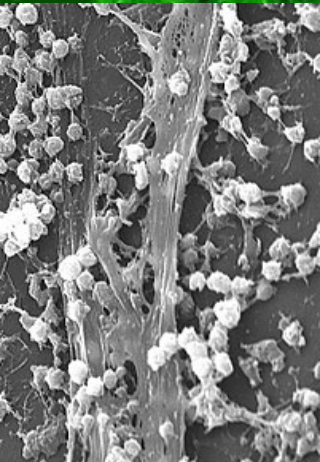
Rawdata

<>	1	2	3	4	5	6	7	8	9	10	11	12	
A	0.9840	0.9040	0.9270	0.4900	0.5470	0.5610	0.5880	0.5310	0.5170	0.6790	0.6270	0.5080	Biofilme
B	0.1650	0.3070	0.1280	0.0970	0.0860	0.1630	0.1380	0.3230	0.4000	0.1140	0.1700	0.1440	
C	0.0980	0.0970	0.0960	0.0940	0.0700	0.0770	0.1110	0.0850	0.1080	0.0830	0.0660	0.1980	
D	0.3650	0.2510	0.2620	0.2810	0.2500	0.2120	0.3040	0.4350	0.3920	0.2530	0.3010	0.4030	Planctónicos
E	0.3720	0.1810	0.2210	0.2200	0.1880	0.1900	0.3030	0.1620	0.1100	0.3320	0.3200	0.3770	
F	0.3510	0.3470	0.3100	0.2710	0.1930	0.3100	0.1780	0.2790	0.3760	0.2540	0.4530	0.3760	
G													
H													
	Controlo			Cloranfenicol			Canamicina			Rifampicina			

Rawdata

<>	1	2	3	4	5	6	7	8	9	10	11	12	
A	0.7460	0.9010	0.8590	0.6760	0.6410	0.5420	0.5700	0.6240	0.5430	0.4890	0.6780	0.5470	Biofilme
B	0.5700	0.0860	0.1220	0.1660	0.0920	0.1200	0.1180	0.0860	0.0990	0.1090	0.0920	0.1240	
C	0.1180	0.0840	0.1520	0.1120	0.2170	0.2600	0.1270	0.1450	0.1990	0.1420	0.0730	0.4110	
D	0.2730	0.2790	0.2930	0.2120	0.2090	0.3210	0.3170	0.2440	0.3920	0.4250	0.2540	0.4510	Planctónicos
E	0.2200	0.2170	0.1450	0.2080	0.1680	0.1710	0.1810	0.2980	0.1360	0.2560	0.2660	0.3560	
F	0.4050	0.2320	0.2170	0.2460	0.2000	0.1330	0.2040	0.2250	0.1390	0.4740	0.3530	0.4000	
G													
H													
	Controlo			Cloranfenicol			Canamicina			Rifampicina			





mg	Abs.
0	0.037
0.5	0.239
1	0.444
2	1.085
3	1.315
4	1.644
5	1.929