Il biofilm microbico nelle infezioni croniche

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Hot Spots for Germs

Smartphones: Think of All the Places They've Been (and What We Touch Before Using Them)

The smartphone screen was found at reach 254.9 CFU/cm^2 (10 times more germs than found on a standard toilet seat)

94.5 % of the phones were contaminated with some kind of bacteria, many of which were resistant to multiple antibiotics. *S. aureus* strains isolated from mobile phones of 52% and those strains isolated from hands of 37.7% were methicillin resistant.

[Ulger et al, 2009]

Your Office, Along With Other Communal Surfaces, Are Likely Teeming With Germs

4800 swabs from surfaces in office buildings, bathroom door handles to photocopier buttons to communal microwaves, refrigerators, and coffee pots

The average desktop harbours 20,961 germs per square inch and that's in addition to 3,295 on the keyboard and 1,676 on a mouse and a staggering 25,127 on the phone.

The typical office desk harbours more than 10 million bacteria
(400 times more germs than found on a standard toilet seat)

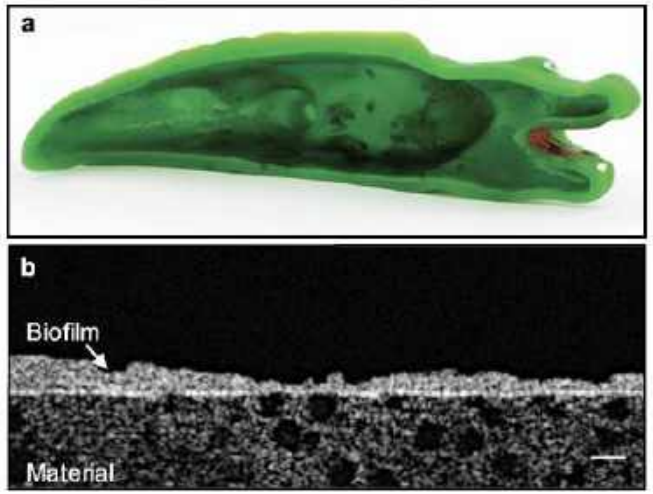
[Mendell et al, 2002]

Ugly ducklings—the dark side of plastic materials

The average total bacterial number was 9.5 x 10^6 CFU/cm2.
(400 000 times more germs than found on a standard toilet seat)

Bacterial community compositions showed the presence of many rare taxa in real bath toys.

Fungi were identified in 58% of all real bath toys.



[Neu et al, 2018]

Do you really want me to shake your hand ?

There are as many as 1,000 resident bacteria per cm^2 on a hand

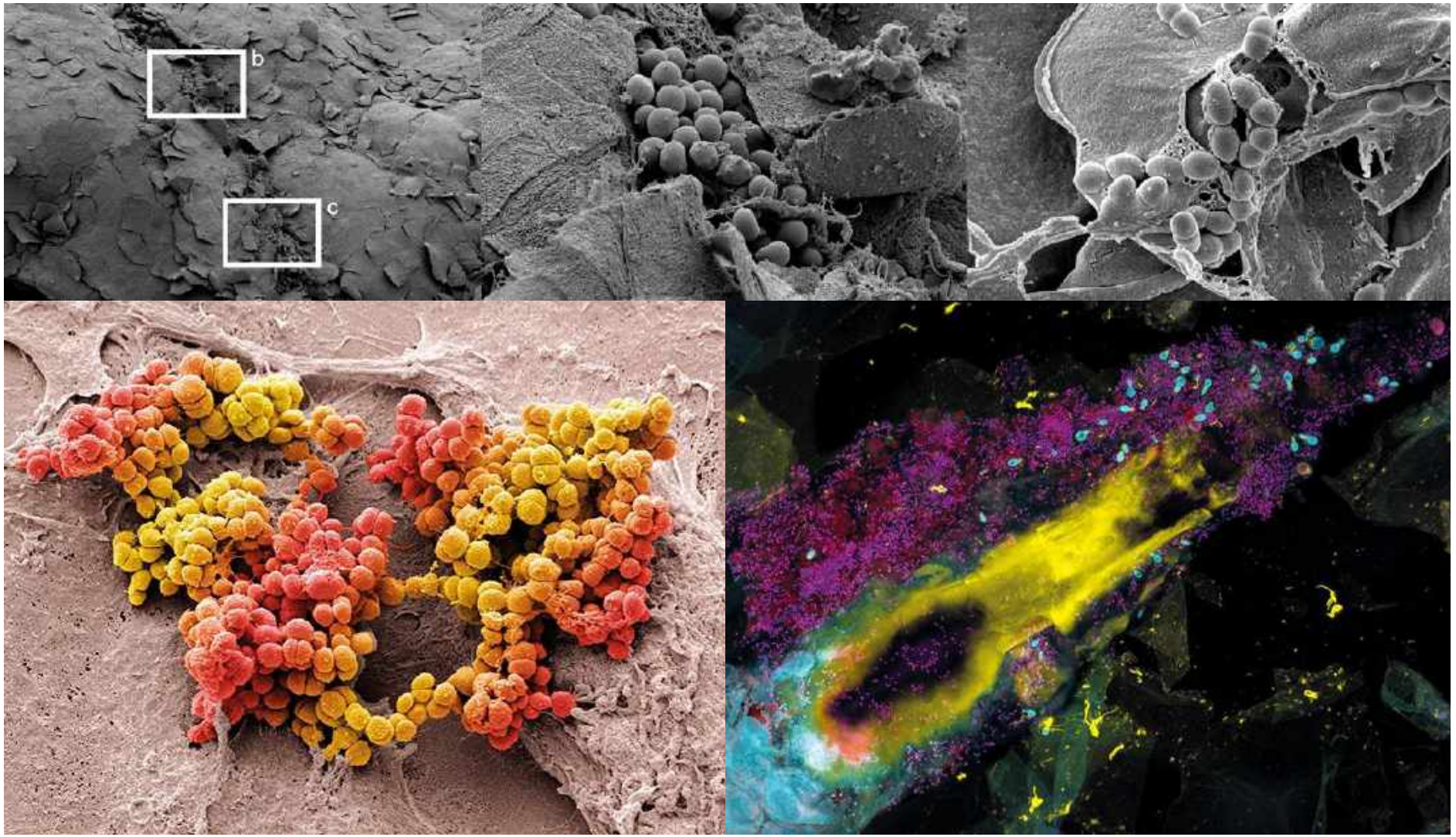
The average hand harboured 150 species of bacteria

The left and right hands of the same individual shared only about 17 per cent of the same bacteria types

The palms of women had a more diverse bacterial composition than those of men

[Hospodsky et al, 2014; Mela and Whitworth, 2014]

How do they live?



The Biofilm theory

How Bacteria Stick

In nature (but not in laboratory cultures) bacteria are covered by a "glycocalyx" of fibers that adhere to surfaces and to other cells. Adhesion might be prevented by a new kind of antibiotic

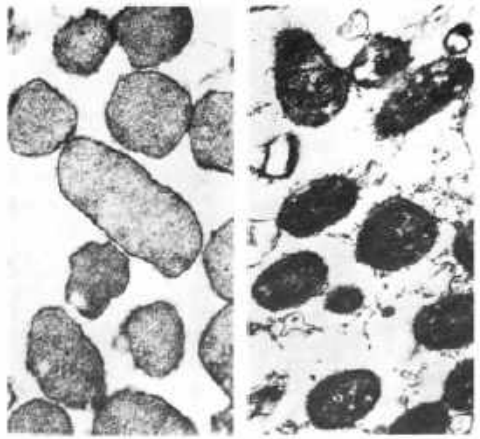
by J. W. Costerton, G. G. Geesey and K.-J. Cheng

Bacteria stick, tenaciously and often with exquisite specificity, to surfaces ranging from the human tooth or lung and the intestine of a cow to a rock submerged in a fast-moving stream. They do so by means of a mass of tangled fibers of polysaccharides, or branching sugar molecules, that extend from the bacterial surface and form a feltlike "glycocalyx" surrounding an individual cell or a colony of cells. The adhesion mediated by the glycocalyx determines particular locations of bacteria in most natural environments; more specifically, it is a major determinant in the initiation and progression of bacterial diseases ranging from dental caries to pneumonia.

These major—and, with the benefit of hindsight, rather obvious—facts about the bacterial cell surface have become known only within the past decade. Ironically the main reason for the late discovery of the bacterial glycocalyx and its functions was the long reliance by microbiologists on an otherwise eminently effective investigative system: the pure laboratory culture of an individual bacterial strain. To generate and maintain a glycocalyx a bacterial cell must expend energy, and in the protected environment of a pure culture the glycocalyx is a metabolically expensive luxury conferring no selective advantage; cells that fabricate these elaborate coatings are usually eliminated from pure cultures by uncoated mutants that can devote more of their energy budget to proliferation. Microbiologists have largely studied such naked mutants.

In a competitive natural environment populated by several kinds of bacteria, on the other hand, selection favors cells that are protected, and enabled to adhere to a desirable surface, by a glycocalyx. It was only in 1969 that Ivan L. Roth of the University of Georgia demonstrated carbohydrate fibers surrounding bacteria in an aquatic system and Ian W. Sutherland of the University of Edinburgh Medical School characterized the surface polysaccharides of bacteria taken from natural environments, thus drawing attention to the universality of what we now know as the glycocalyx. Since then studies in our laboratories at the University of Calgary and at the Agriculture Canada Research Station at Lethbridge in Alberta, and in a number of other laboratories, have made it clear that the glycocalyx is essential to the biological success of most bacteria in most of the varied natural environments in which they are observed.

The polysaccharide-coated surface is not a peculiarity of the bacterial cell. The more rigid polysaccharide cell wall of higher plants was among the first microscopic structures described by Robert Hooke in 1665. The analogous surface of animal cells—a glycocalyx like that of bacteria—was described only in 1971, by Vincent T. Marchesi and his colleagues at the National Institute of Arthritis, Metabolism, and Digestive Diseases. They isolated and identified glycoproteins arrayed in the membrane of animal cells and showed that the polysaccharide fibers they bear extend outward from the membrane to form

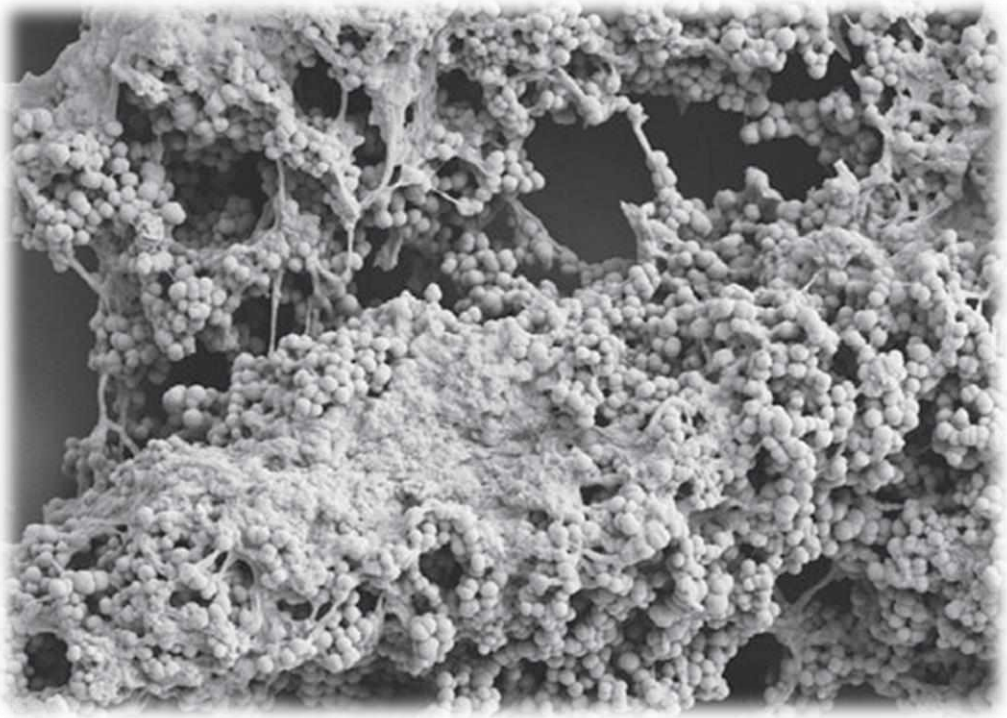


NAKED BACTERIA (*left*) are from a typical pure laboratory culture of *Escherichia coli*; the glycocalyx-coated bacteria (*right*) are *Pseudomonas* cells from an infected human bladder. In both preparations the cells were stained with ruthenium red, which is taken up by any polysaccharide glycocalyx fibers that are present. The bacterial glycocalyx was ignored until recently because the familiar pure laboratory strains do not need it and therefore do not fabricate it.

In 1978 Bill Costerton warned that chronic infections in patients with indwelling medical devices were caused by biofilm bacteria and that bacteria within biofilms resist antibiotic therapies and immune host defenses

What is a Biofilm?

A biofilm is made up of colonies of one or more type of microorganisms that form together under a sticky glue-like substance and attach to all kinds of surfaces. Microorganisms such as bacteria and fungi, commonly make up biofilm colonies.



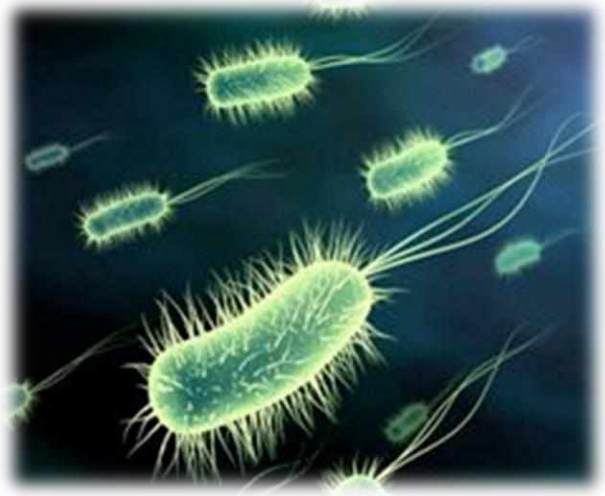
Biofilm cells are frequently embedded within a self-produced matrix of extracellular polymeric substance (EPS).

Biofilm EPS, is a polymeric jumble of DNA, proteins and polysaccharides.

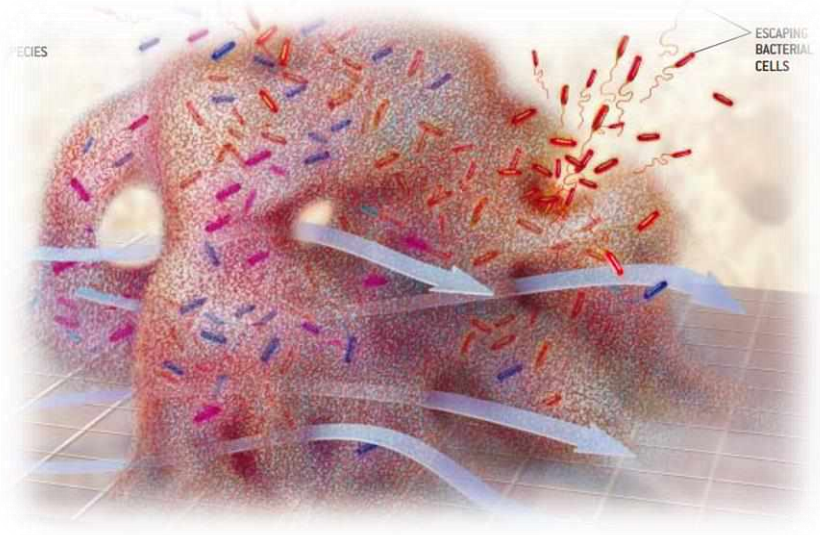
Biofilms: The social life of microorganisms

Biofilms formation provides significant benefits in terms of host colonization, defence against competitors and adaptation to changing environments

Planktonic bacteria



Bacterial biofilm



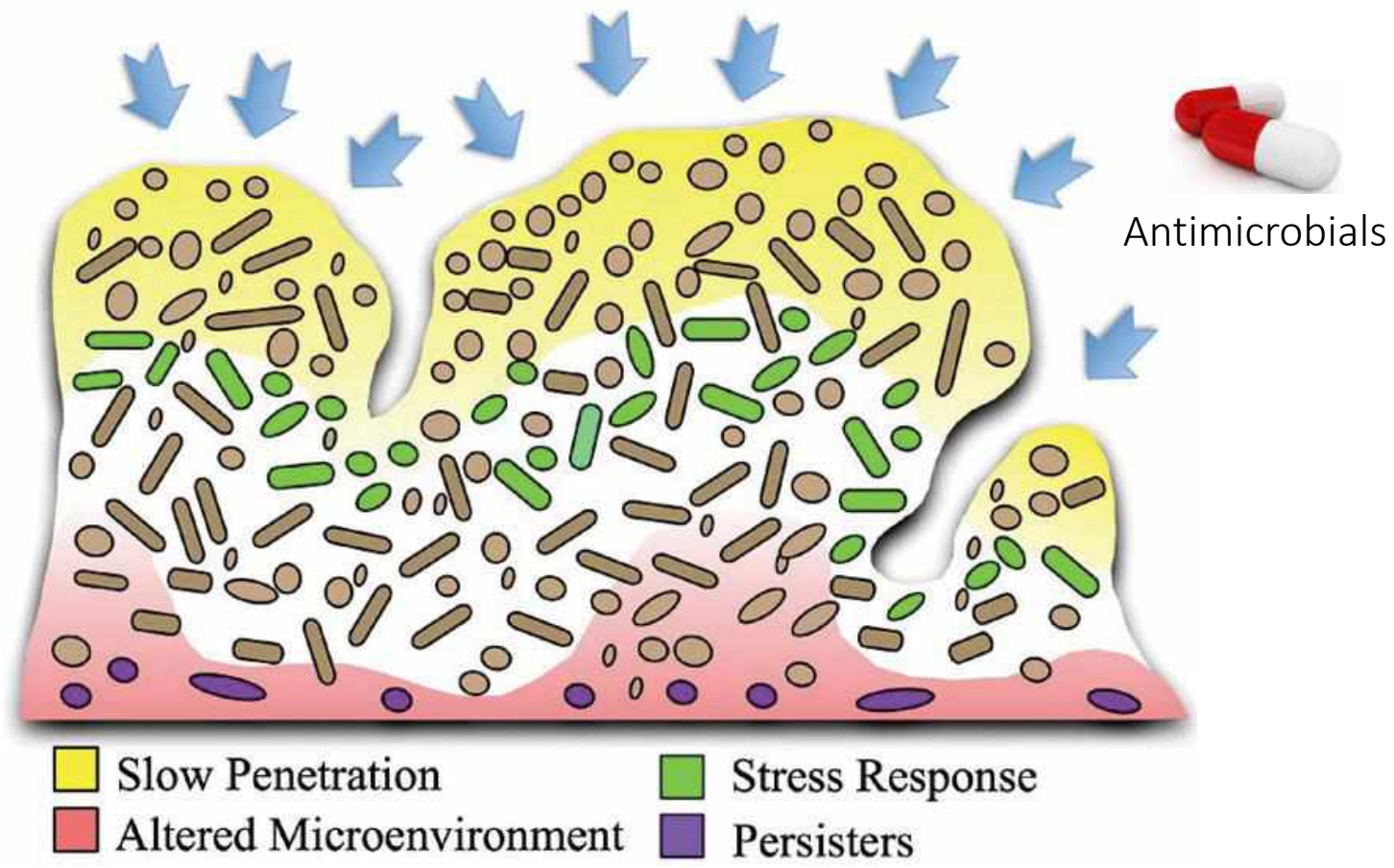
Extracellular matrix

Growth rates

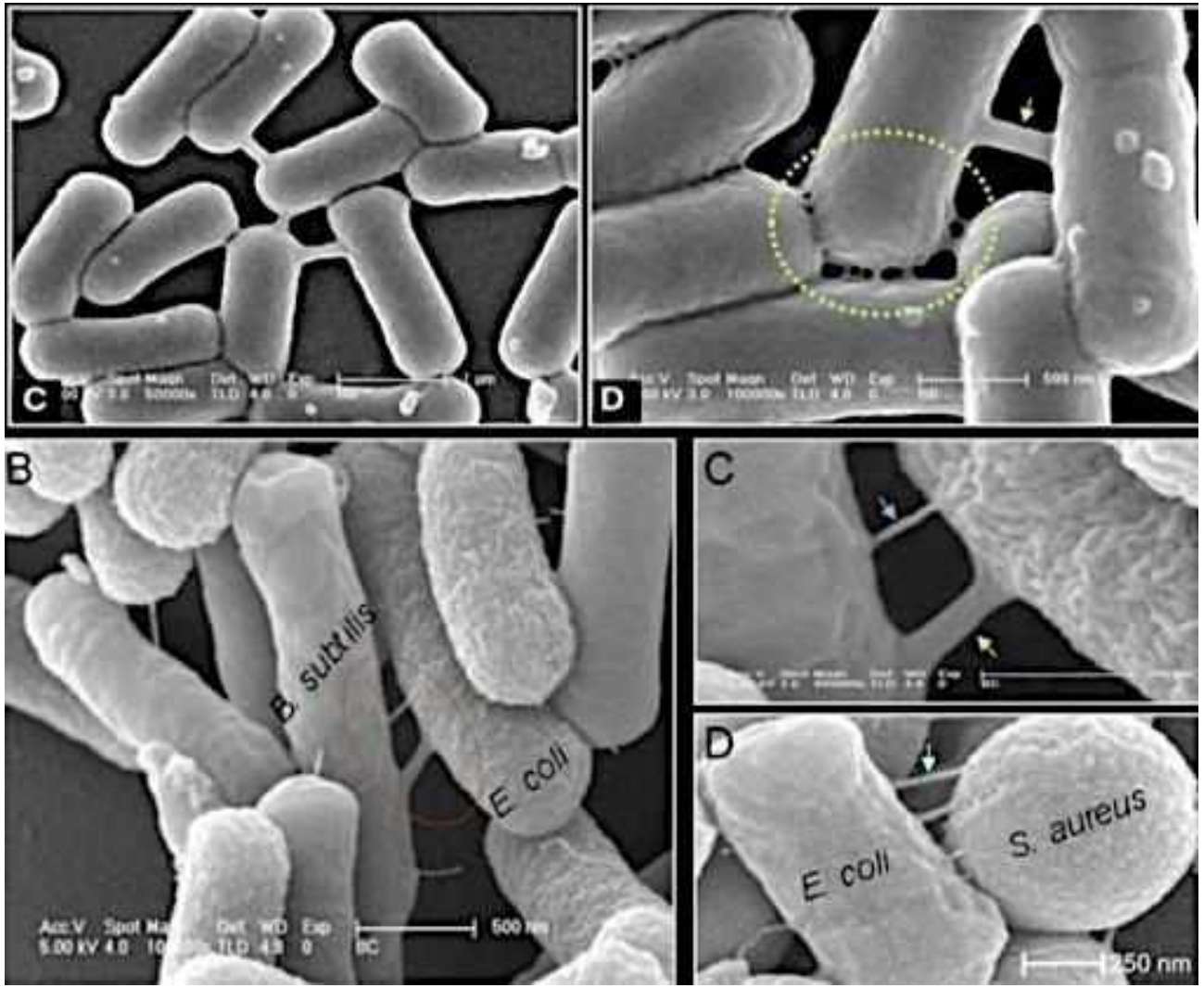
Gene expression

Biofilm provides increased antimicrobial tolerance

Bacteria living in biofilms can be up to 1,000/1500 times more tolerant to antibacterial compounds than their planktonic counterparts



Biofilm production and multidrug resistance (MDR)



Horizontal gene transfer: conjugation, transformation, transduction are increased in biofilms

The biofilm lifestyle also increases plasmid stability and the range of mobile genetic elements

Distinguishing between resistance, tolerance and persistence

Resistance

The genetic ability to counteract antimicrobial treatments.
Resistance is quantified by the minimum inhibitory concentration (MIC)

Tolerance

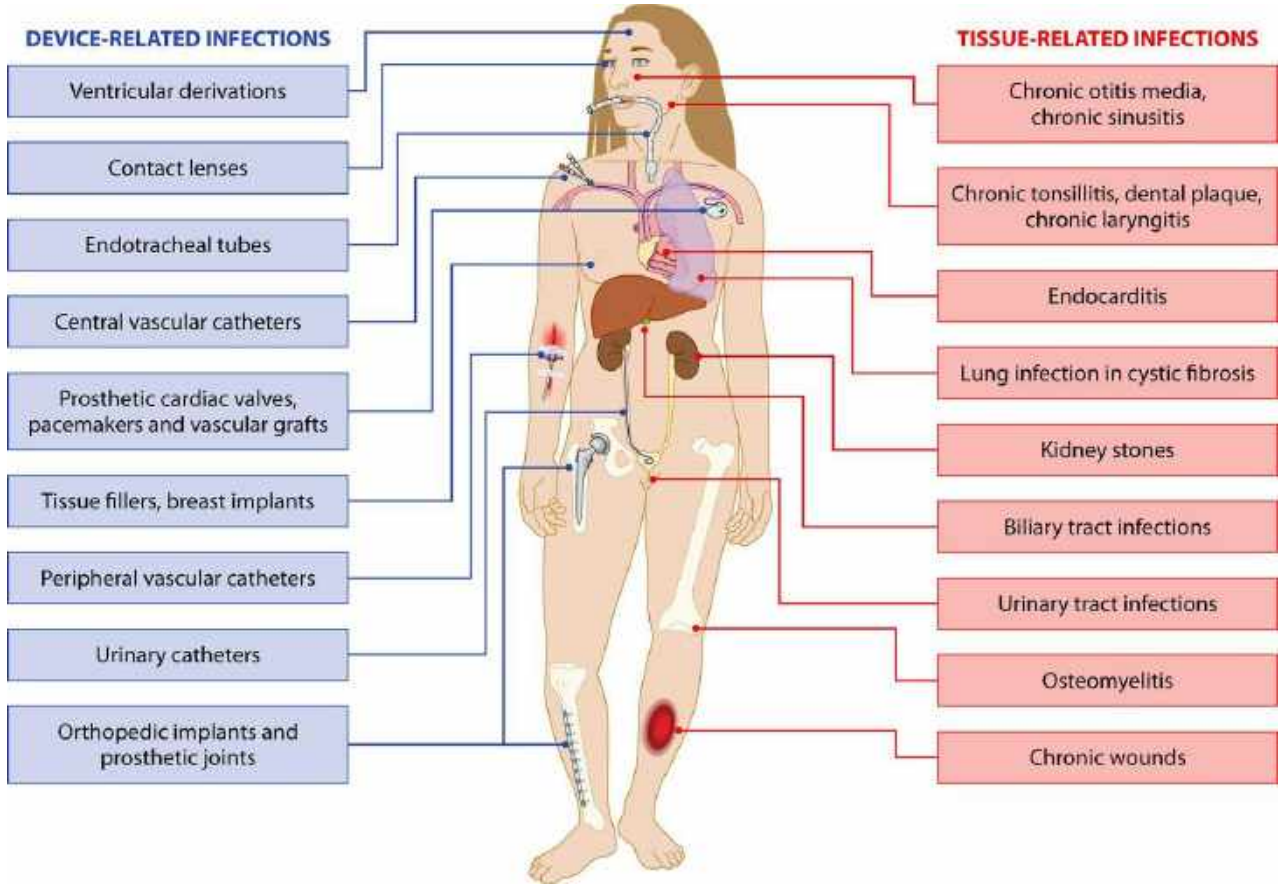
The ability to evade antimicrobial treatments of genetically susceptible microorganisms.
Tolerance can be quantified by the minimum duration for killing (MDK99).

Persistence

Prolongs the duration of treatment that bacteria can sustain only for a subpopulation (MDK99.99)

Clinical relevance of Biofilm infections

Biofilms are present in more than 80% of all human infections

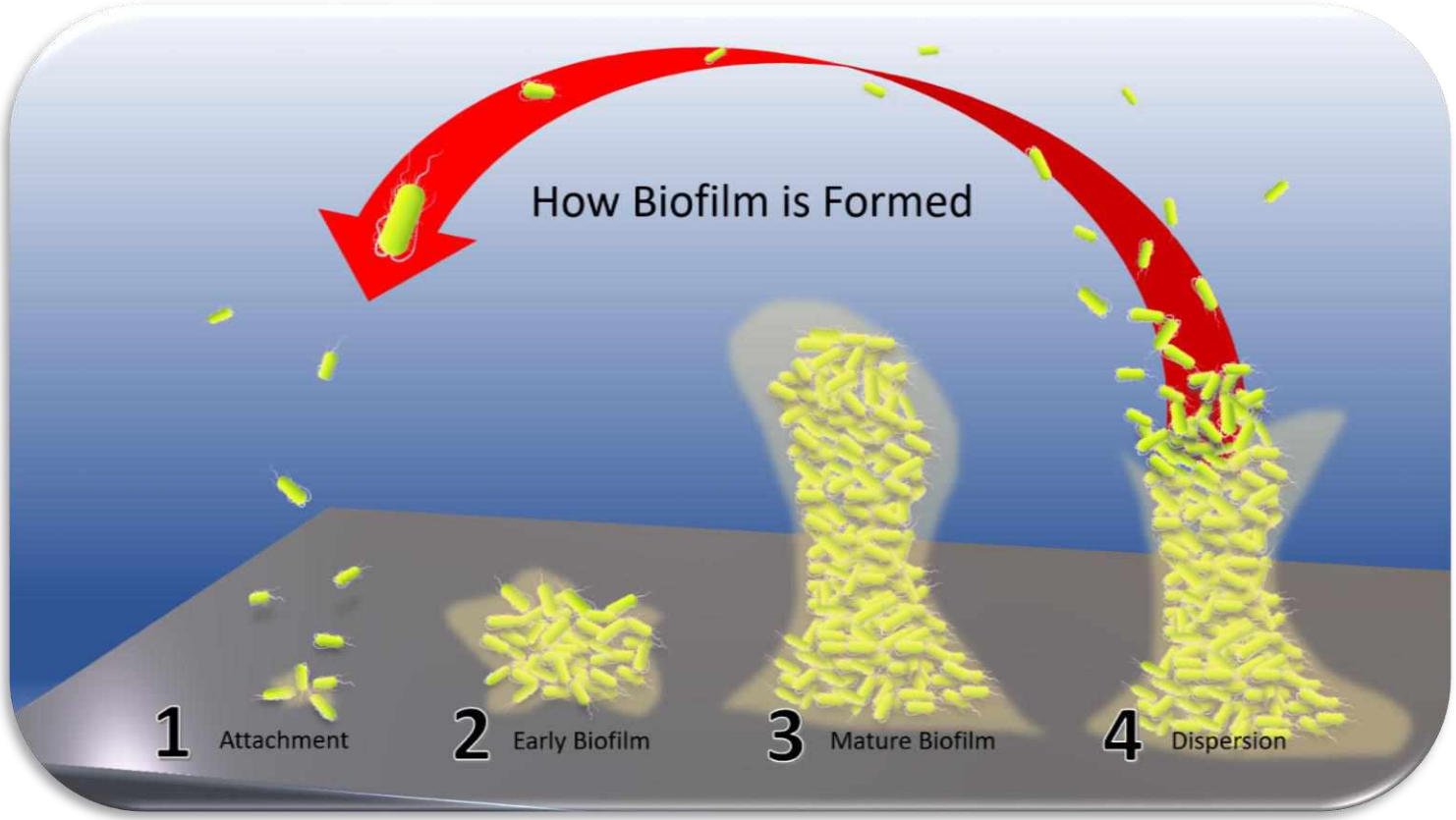


Biofilms cause over 2 million infections annually, resulting in US$11B in additional costs

[Lebeaux et al. 2014, *Microbiol Mol Biol Rev*. 78, : 510–543]

What is the optimal antibiotic strategy for treatment of established biofilm infections?

Antibiograms are performed on planktonic cells and do not take into account biofilm



The opportunity


frontiers in Neurology

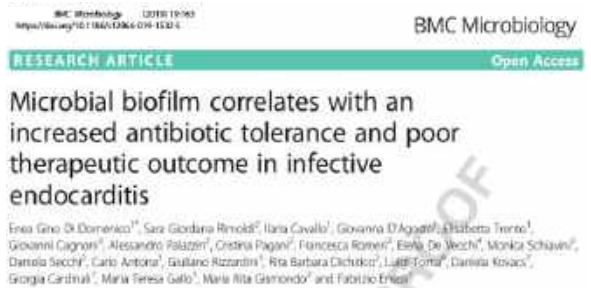
The Emerging Role of Microbial Biofilm in Lyme Neuroborreliosis


frontiers in Microbiology

Development of an *in vitro* Assay, Based on the BioFilm Ring Test®, for Rapid Profiling of Biofilm-Growing Bacteria


SCIENTIFIC REPORTS nature

Inflammatory cytokines and biofilm production sustain *Staphylococcus aureus* outgrowth and persistence: a pivotal interplay in the pathogenesis of Atopic Dermatitis


BMC Microbiology

RESEARCH ARTICLE Open Access

Microbial biofilm correlates with an increased antibiotic tolerance and poor therapeutic outcome in infective endocarditis


International Journal of Molecular Sciences MDPI

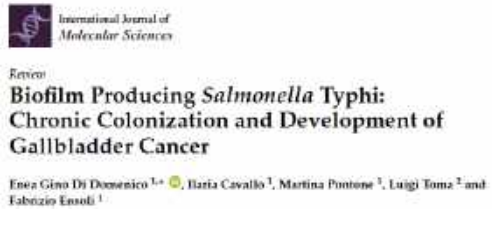
Article

Biofilm is a Major Virulence Determinant in Bacterial Colonization of Chronic Skin Ulcers Independently from the Multidrug Resistant Phenotype


FEMS JOURNALS

RESEARCH ARTICLE

The clinical Biofilm Ring Test: a promising tool for the clinical assessment of biofilm-producing Candida species


International Journal of Molecular Sciences

Review

Biofilm Producing *Salmonella* Typhi: Chronic Colonization and Development of Gallbladder Cancer


MDPI

Antimicrobial Resistance and Infection Control

RESEARCH Open Access

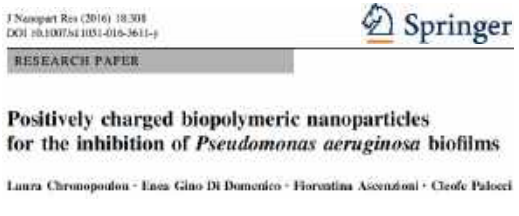
Ralstonia mannitolilytica infections in an oncologic day ward: description of a cluster among high-risk patients


International Journal of Pharmaceutics

Nanometric ion pair complexes of tobramycin forming microparticles for the treatment of *Pseudomonas aeruginosa* infections in cystic fibrosis

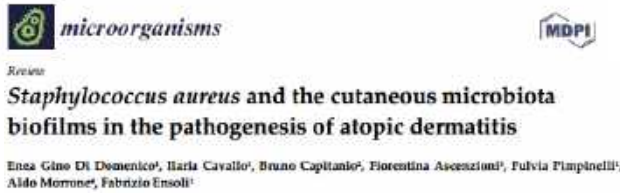

MAGNETIC RESONANCE IN MEDICINE ISMRM ONE

A Novel Approach Based on Low-Field NMR for the Detection of the Pathological Components of Sputum in Cystic Fibrosis Patients


Springer

RESEARCH PAPER

Positively charged biopolymeric nanoparticles for the inhibition of *Pseudomonas aeruginosa* biofilms


microorganisms MDPI

Review

Staphylococcus aureus and the cutaneous microbiota biofilms in the pathogenesis of atopic dermatitis

ISG IRE

clinical BIOFILM RING TEST® (cBRT)

The working protocol

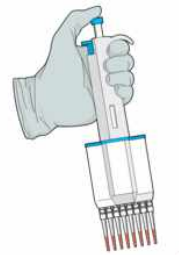
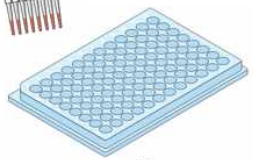


Plate preparation
(11 strains)



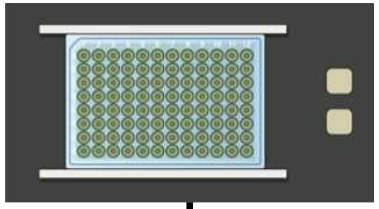


Plate analysis

20 minutes

5 hours

10 minutes

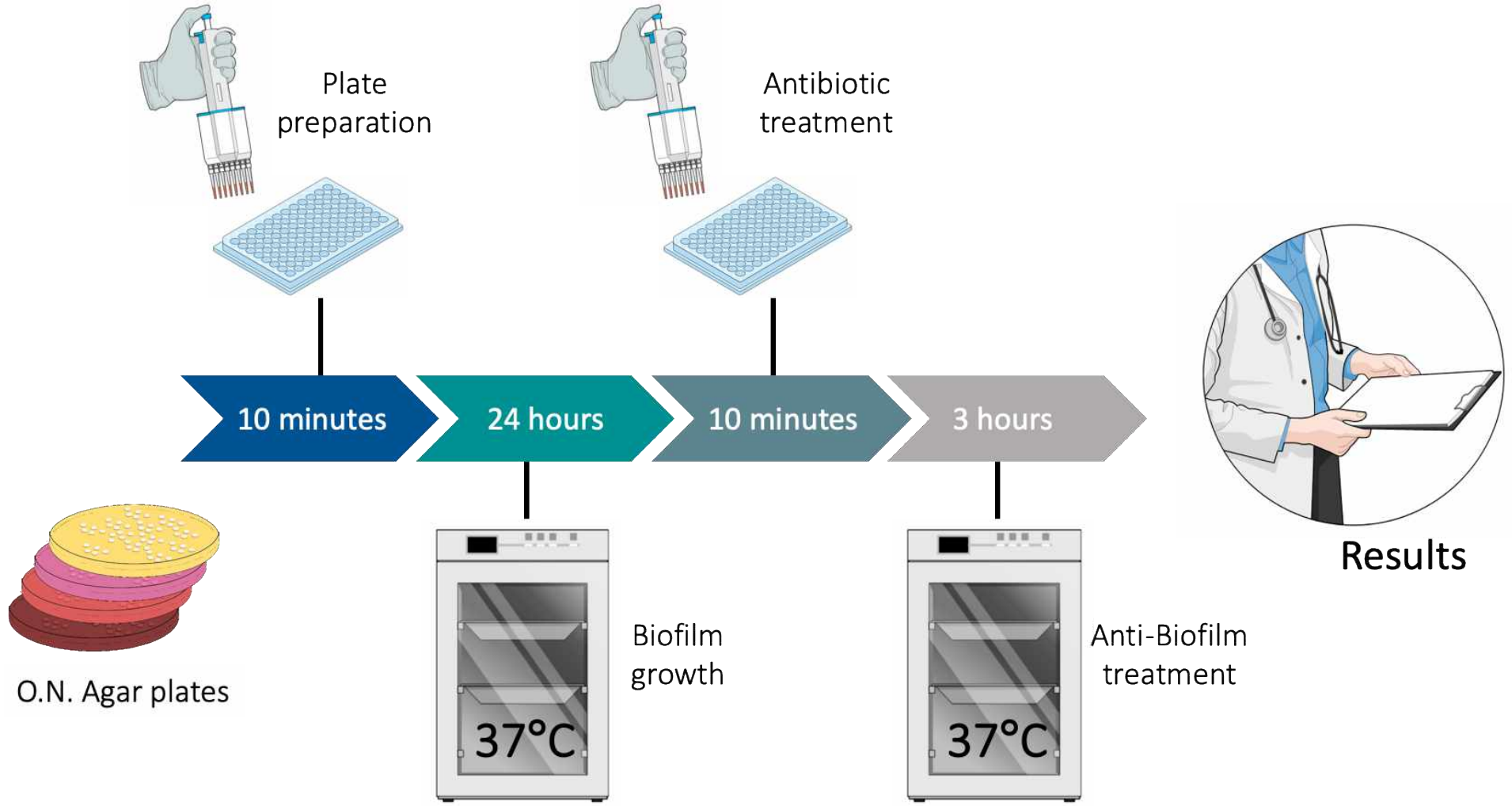
O.N. Agar plates

Incubation

Results

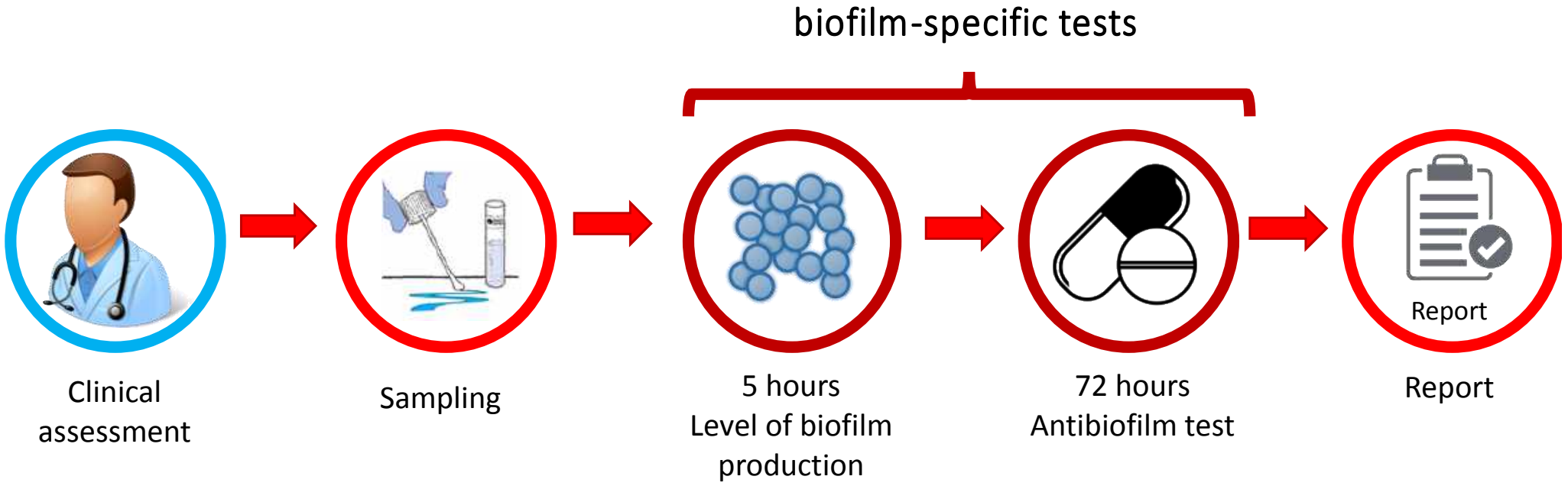
Patent number WO2017/1219461

Biofilm Antimicrobial Susceptibility Testing (BiofAST)

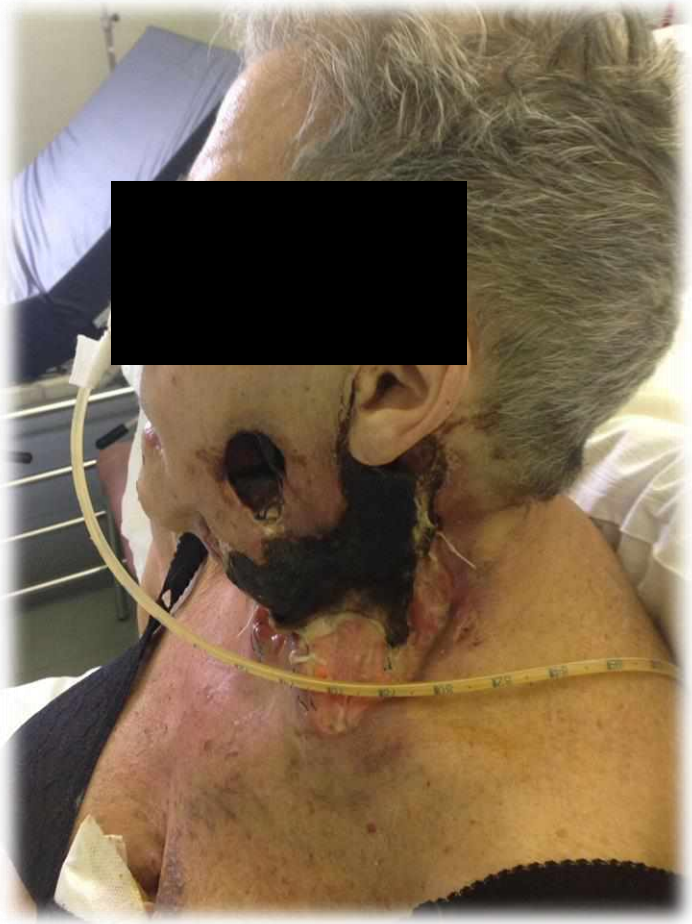


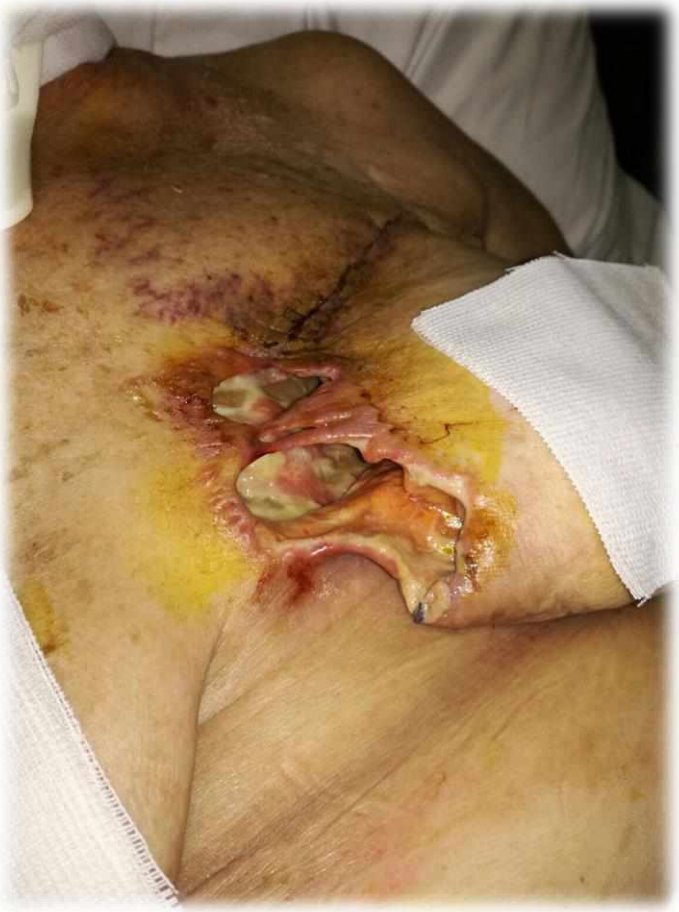
Patent application N: 102019000013983

Development of innovative strategies for the treatment of biofilm infections



Clinical and microbiological aspects of biofilm-associated surgical site infections





Case presentation

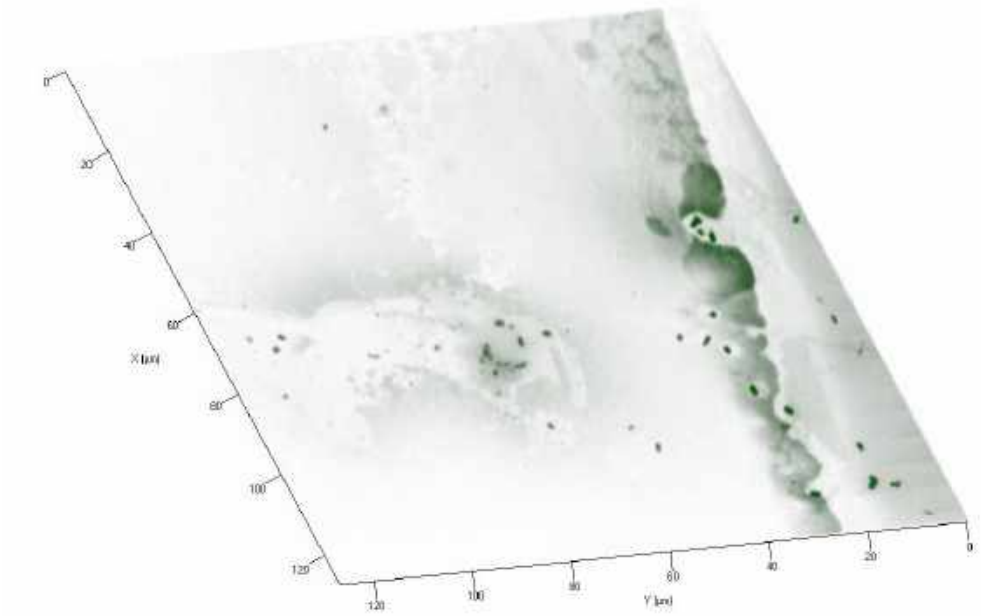
78-Year-old woman with oral cancer

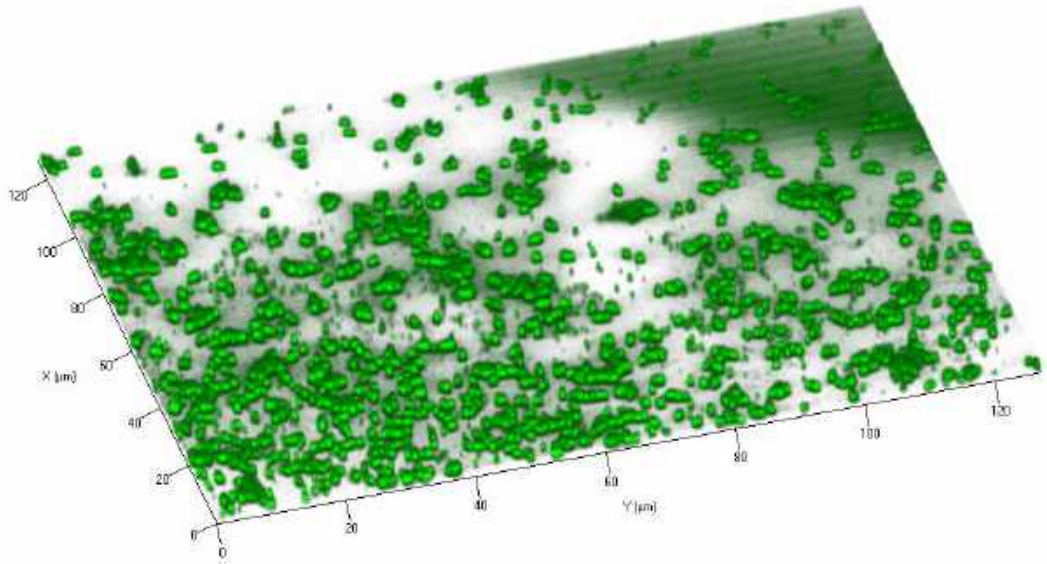
Tracheostomy over the second tracheal ring

Pectoralis major flap for reconstructive head and neck surgery

Surgical site infection by:
Staphylococcus aureus
Pseudomonas aeruginosa

Biofilm on suture





Co-infection by *S. aureus* and *P. aeruginosa*

I.D.: XXX Sig. **XXX** | Sesso F | MICROBIOLOGIA

Data di Nascita: XXX | Età: 78 Anni | PROVENIENZA: OTORINO | Materiale: **TAMPONE ULCERA**

STRAIN: ***Staphylococcus aureus*** | ***Pseudomonas aeruginosa***

RESULT: **Moderate biofilm producer** | **High biofilm producer**

Antimicrobials	MIC (mg/L)	INT	BMIC (mg/L)	INT
Benzilpenicillin	> 0.5	R	> 8	R
Clindamycin	≤ 0. 25	S	> 2	R
Daptomycin	≤ 0. 50	S	4	R
Erythromycin	> 2	R	4	R
Fusidic Acid	≤ 0,5	S	≤ 1	S
Gentamicin	≤ 1	S	1	S
Linezolid	2	S	1	S
Oxacillin	> 2	R	> 2	R
Rifampicin	-	-	≤ 0.06	S
Teicoplanin	≤ 0,5	S	4	R
Tigecycline	0.25	S	0.25	S
TMP/SMX	≤ 10	S	≤ 10	S
Vancomycin	≤ 0,5	S	2	S

EUCAST Clinical breakpoints - bacteria (v 9.0)

Antimicrobials	MIC (mg/L)	INT	BMIC (mg/L)	INT
Amikacin	≤ 2	S	≤ 2	S
Cefepime	≤ 1	S	> 32	R
Ceftazidime	4	S	32	R
Ciprofloxacin	≤ 0.25	S	> 2	R
Gentamicin	≤ 1	S	1	S
Imipenem	2	I	> 16	R
Meropenem	1	S	> 16	R
PIT	8	S	> 128	R

Piperacillin/Tazobactam (PIT)
TMP/SMX = Trimethoprim/Sulfamethoxazole

Daptomycin
+
Meropenem

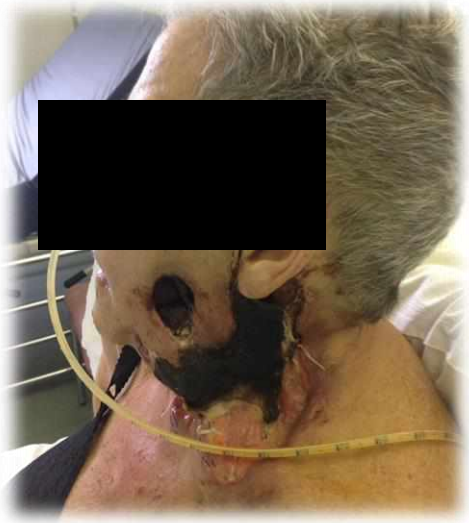
Gentamicin
+
Meropenem

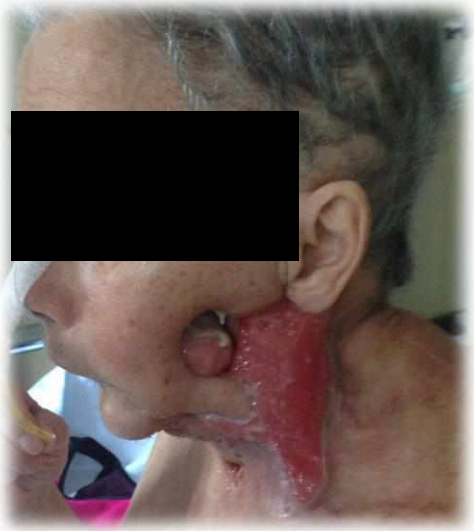
D=0

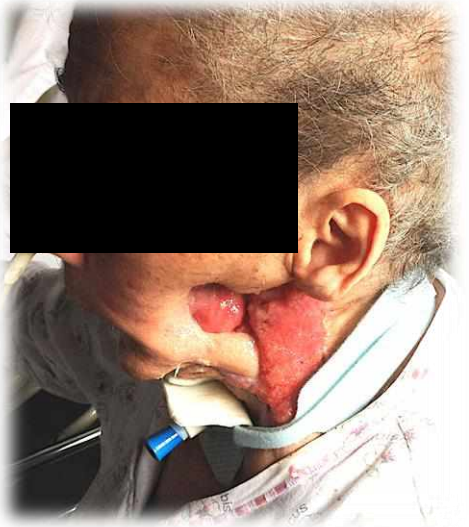
D=7

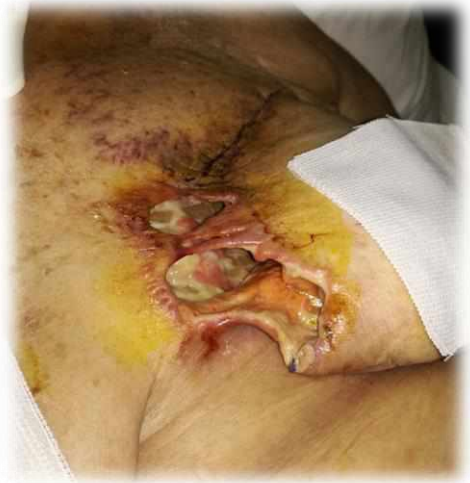
D=14

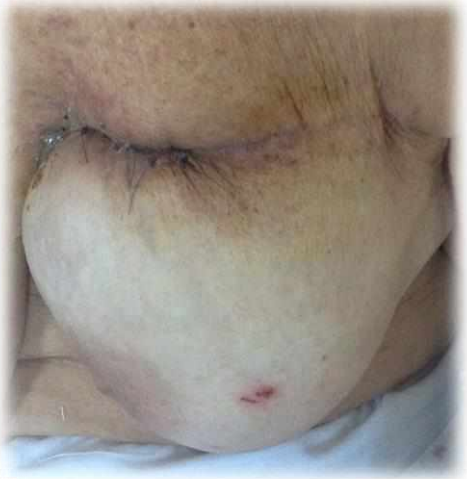
D=21

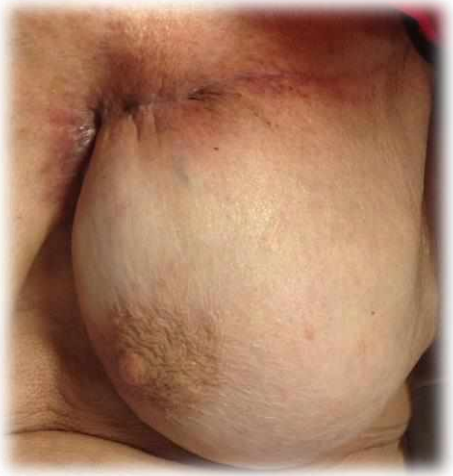












Conclusions

There are not many answers about how to treat chronic biofilm infections

The strength of biofilm production is a key risk factor affecting the antimicrobial therapy

Early, aggressive and targeted antibiotic treatments against biofilm infections

Biofilm profiling with cBRT and antibiotic efficacy on biofilm-growing microorganisms with BiofAST represent promising tools to predict the clinical outcome of antibiotic therapy

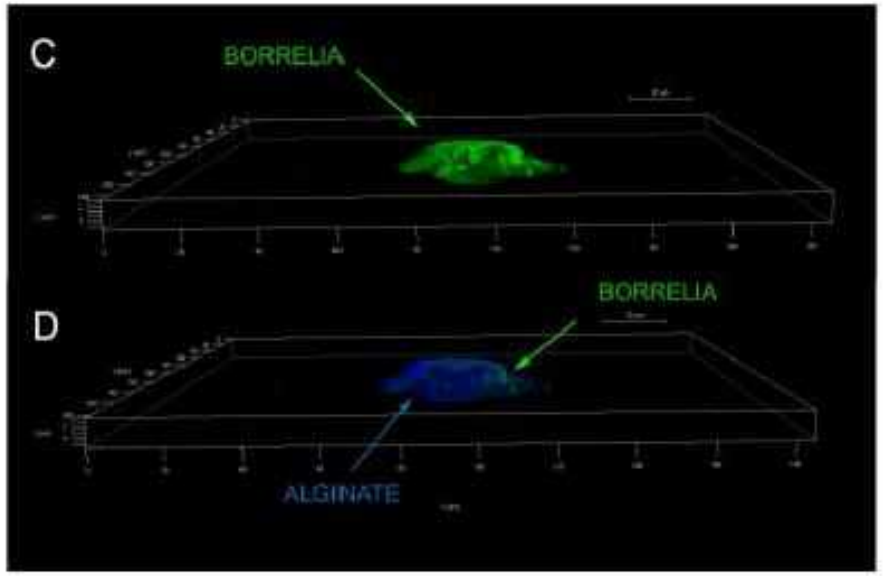
Biofilm Production in *Borrelia burgdorferi*: *in vivo* and *in vitro* evidences

Article

The Long-Term Persistence of *Borrelia burgdorferi* Antigens and DNA in the Tissues of a Patient with Lyme Disease

Eva Sapi [1,*], Rumanah S. Kasliwala [1], Hebo Ismail [1], Jason P. Torres [1], Michael Oldakowski [1], Sarah Markland [1], Gauri Gaur [1], Anthony Melillo [1], Klaus Eisendle [2], Kenneth B. Liegner [3,4,5], Jenny Libien [6] and James E. Goldman [7]

CONFOCAL MICROSCOPY

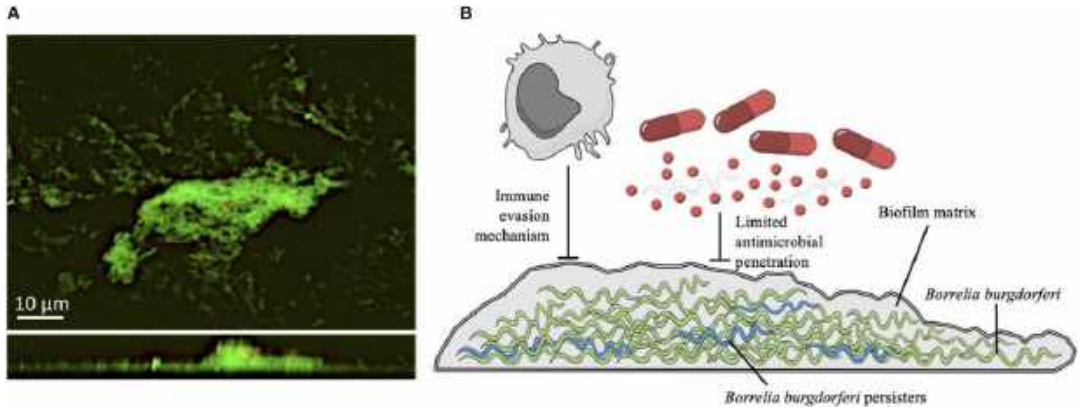


Borrelia biofilm in infected liver tissue

frontiers in Neurology

The Emerging Role of Microbial Biofilm in Lyme Neuroborreliosis

Enea Gino Di Domenico[*], Ilaria Cavallo[1], Valentina Bordignon[1], Giovanna D'Agosto[1], Martina Pontone[1], Elisabetta Trento[1], Maria Teresa Gallo[1], Grazia Prignano[1], Fulvia Pimpinelli[1], Luigi Toma[2] and Fabrizio Ensoli[1]



Thank you

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ISG ISTITUTO DERMATOLOGICO SAN GALLICANO

ISTITUTI DI RICOVERO E CURA A CARATTERE SCIENTIFICO

Microbiology and Virology
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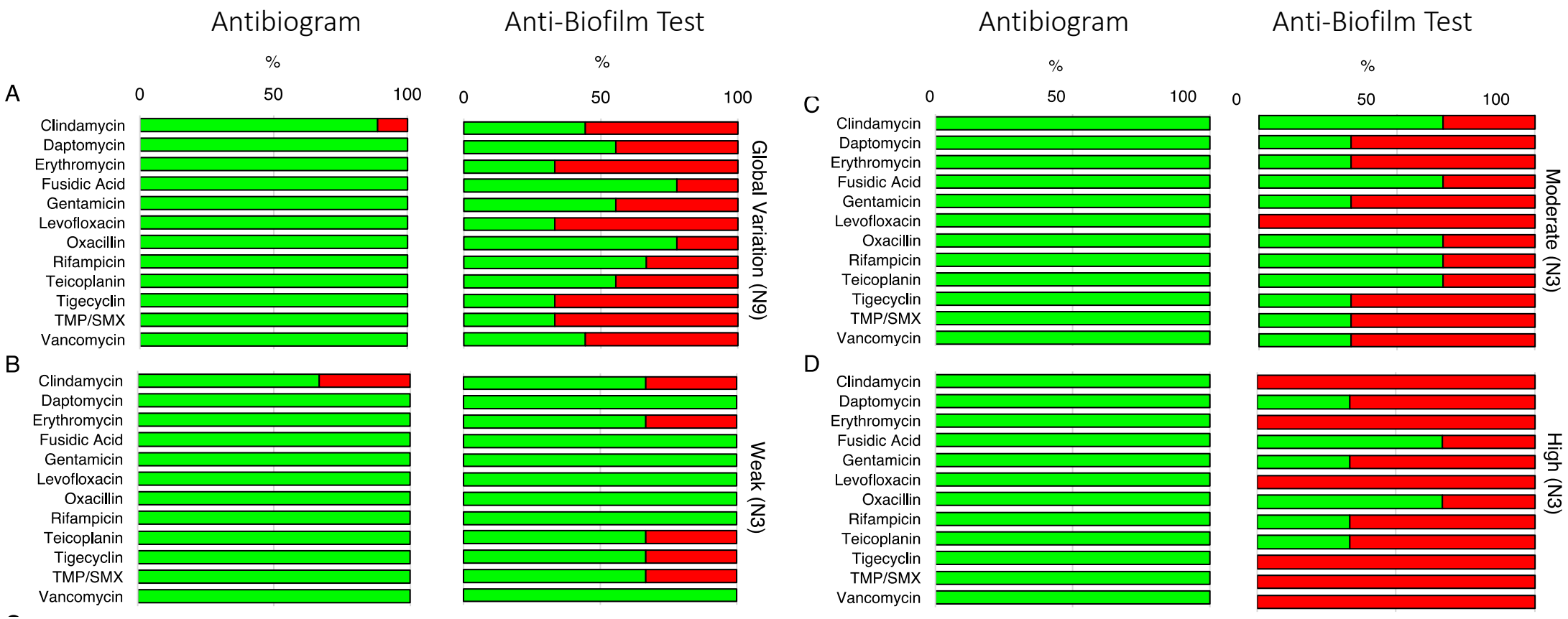
email: enea.didomenico@ifo.gov.it

Anti-Biofilm Test:
the antibiotic profiling on biofilm-producing bacteria



Antibiotic tolerance is linked to the level of biofilm production