BIOFILM INFECTIONS: A HISTORICAL PERSPECTIVE

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ABSTRACT

Antony van Leeuwenhoek (1632-1723) was the first to observe and describe in the late 1680s aggregated microbes adhering to surfaces of teeth and tongue, what we today call biofilms. Although observed and described for more than 300 years ago, the recognition of their importance in medicine is, however, less than 40 years old. They were first observed by Høiby as heaps of *P. aeruginosa* cells in sputum and lung tissue from chronically infected cystic fibrosis patients and by Jendresen as acquired dental pellicles. John William Costerton (1934-2012) was though the one who introduced the term biofilm into medicine and he made major contributions for the understanding of their significance in chronic infections and foreign body infections e.g. on medical devices. During the following decades, in parallel with development of new technologies, major achievements have been done in understanding the biology of biofilms. In spite of accumulation of a large amount of knowledge, biofilms still represent a major diagnostic and treatment challenge.

Keywords: Biofilms, microbial biofilms, biofilm infection, foreign body infection, *Pseudomonas aeruginosa*, cystic fibrosis.

REZUMAT

Antony van Leeuwenhoek (1632-1723) a fost primul care a observat și descris în anii 1680 microbii agregați care aderau la suprafața dinților și a limbii, ceea ce astăzi numim biofilme. Deși au fost observate și descrise cu mai bine de 300 de ani în urmă, recunoașterea importanței biofilmelor în medicină are mai puțin de 40 de ani. Acestea au fost observate pentru prima oară de Høiby, sub forma unor grupuri de bacterii *P. aeruginosa* din spută și țesut pulmonar, provenind de la pacienți cu fibroză chistică și infecție pulmonară cronică și Jendresen, sub forma unor pelicule dentare. John William Costerton (1934-2012) a fost cel care a introdus termenul de biofilm în medicină și a contribuit major la înțelegerea semnificației lor în infecțiile cronice și infecțiile produse pe implanturi, de ex. pe catetere intravasculare. În decursul următoarelor decenii, în paralel cu dezvoltarea de noi tehnologii, s-au obținut realizări majore în înțelegerea biologiei biofilmelor. În ciuda acumulării unei cantități mari de cunoștințe, biofilmele reprezintă în continuare o problemă majoră de diagnostic și tratament.

Cuvinte-cheie: biofilme, biofilme microbiene, infecții produse de biofilme, infecții produse de implanturi, *Pseudomonas aeruginosa*, fibroză chistică.

Microorganisms can adopt two main forms of life: either planktonic, free swimming, single bacteria or as microbial aggregates. Microbial cells organized in a structured community and embedded in a self-produced matrix are called biofilms which may be adhering to surfaces or situated in the tissue or in secretions. Both monospecies and polyspecies biofilms exist and components from the host may be found in biofilms.

In nature, bacteria are primarily growing as biofilms, which are the oldest and most successful form of life on Earth with fossils dating back 3.5 billion years and representing the first form of life [1].

These bacterial communities live on soil particles, in rock fissures, marine and river sediments and at the very extremes of terrestrial habitats from inside Antarctic ice to the walls of sea hydrothermal vents.

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Evidence of early association of microbes with surfaces is present in fossil records [2].

The first description of bacterial aggregates dates back in the late 1680es and the beginning of 1700es when Anthony van Leeuwenhoek (1632-1723) from Delft, the Netherlands, the discoverer of the microscope, analyzed the "scurf of the teeth" and "particles scraped off his tongue". "I saw an inconceivable number of exceeding small animalcules and these of diverse sorts. But far the greatest number was of one and the same bigness, yet so little that they could not be discerned but by great attention, through a very good magnifying glass; and most of these animalcules were abiding where the said matter from the tongue lay, and I took into consideration whether the said creatures might not indeed be getting their food from the particles of the tongue." was he writing in his letters to the Royal Society where he described his observations (Figs. 1A, B). He refers to the dental plaque development and the normal flora of the mucosa of the tongue later described by many dentists [3].

Two hundred years later, in 1864, aggregates of bacteria were also observed and sketched by Louis Pasteur who recognized their role in wine becoming acetic, which led to his discovery of pasteurization.

However, the name "biofilm" of this ancient mode of bacterial life was first used between 1920-1930 by marine biologists who studied biofouling and referred to bacterial adhesion, aggregation and multiplication on surfaces to distinguish adhering (sessile) bacteria from free swimming 'planktonic' bacteria. In 1923 Angst found that slime on the ships' bottoms is caused to a large extent by bacteria [4].

In 1933, Henrici published his observation of biofouling in fresh water "it is quite evident that for the most part water bacteria are not free floating organisms, but grow attached upon submerged surfaces" and he illustrated this by a number of drawings in his article [5].

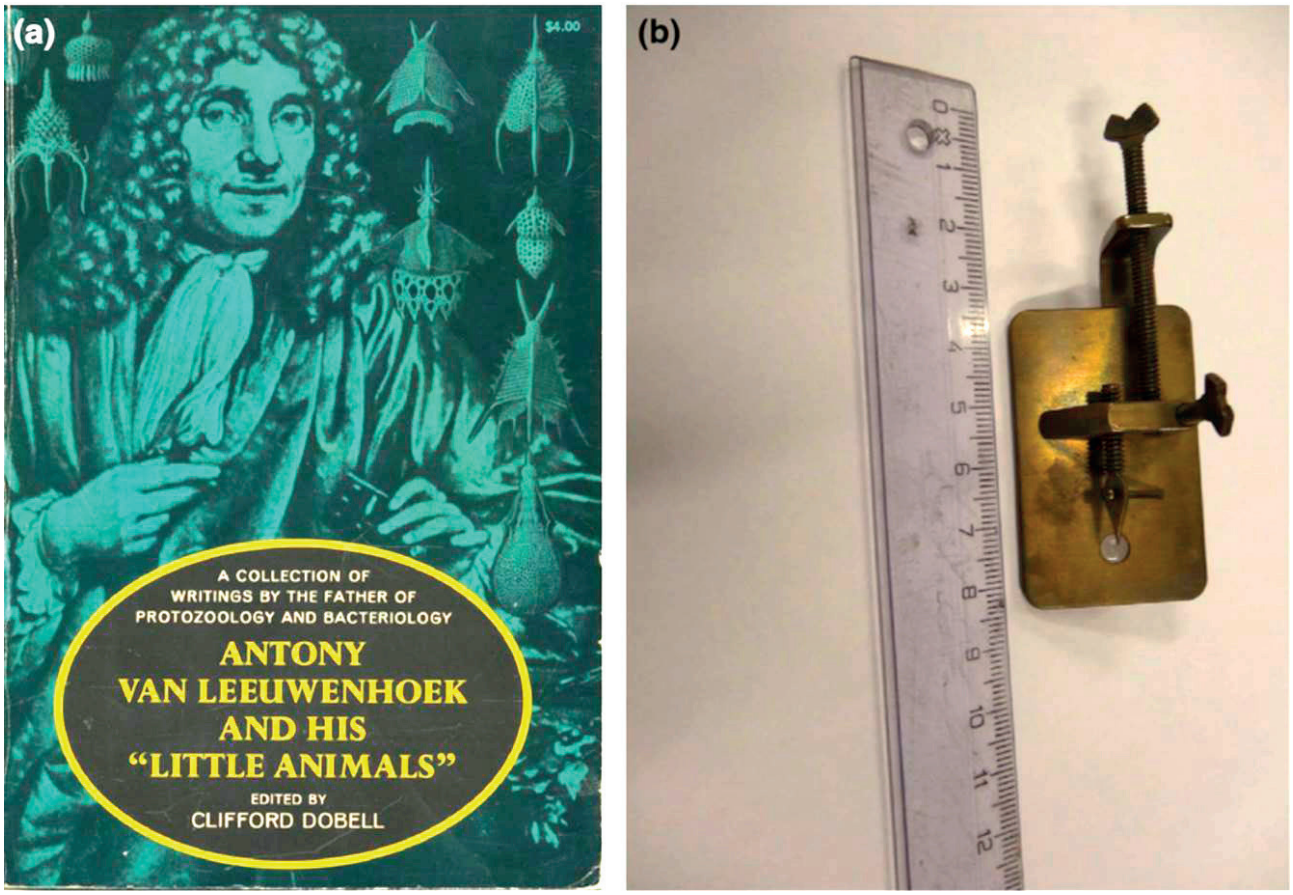

Fig. 1. (a) Antony van Leeuwenhoek (1632–1723) was the first to observe and describe microbial biofilms in his own mouth. **(b)** His 'microscope', a replica bought at an ASM general meeting.

In 1935, ZoBell & Allen from the University of California, published their observation on the early development of biofouling in sea water and they observed on submerged glass-slides that the process was initiated "by biofilm growing bacteria and to a lesser extent, other microorganisms and that such films favor the subsequent attachment of the larger and more inimical fouling organisms. The film of bacteria may promote the attachment of macroscopic organisms in different ways…" [6]. They found, that "ordinarily it requires two to four hours for appreciable numbers of bacteria to become attached solidly to glass slides.… so firmly glued to the slide that running water will not detach them". It is obvious from Fig. 2 that the number of bacteria they counted on the surface of the slides is orders of magnitudes higher than the number of individual bacterial cells free flowing in the water.

However, biofilm growing microorganisms were not of interest and unknown for most microbiologists and the microbiological research especially in medicine was focused successfully on planktonically growing microorganisms and their pathogenic and other properties.

The concept of biofilm infections and their importance in medicine is only 40 years old and was started by Jendresen observations of acquired dental pellicles [7] and by Prof. Niels Høiby (University Hospital, Copenhagen, Denmark) when he observed in early seventies "heaps" of bacteria by routine microscopy of sputum collected from patients with cystic fibrosis and chronic *P. aeruginosa* infection. The first images of such a biofilm were published in 1977 (Fig. 3) and showed aggregated bacteria (heaps) surrounded by abundance of slime were observed in sputum of CF patients chronically infected with mucoid strains of *P. aeruginosa* [8].

In 1978, Prof. John William (Bill) Costerton from the University of Calgary, Alberta, Dept. of Biochemistry and Microbiology, published "How bacteria stick" [9] and stated that "in nature bacteria are covered by a "glycocalyx" of fibers that adhere to surfaces and to other cells. Bacteria stick tenaciously (…) to surfaces

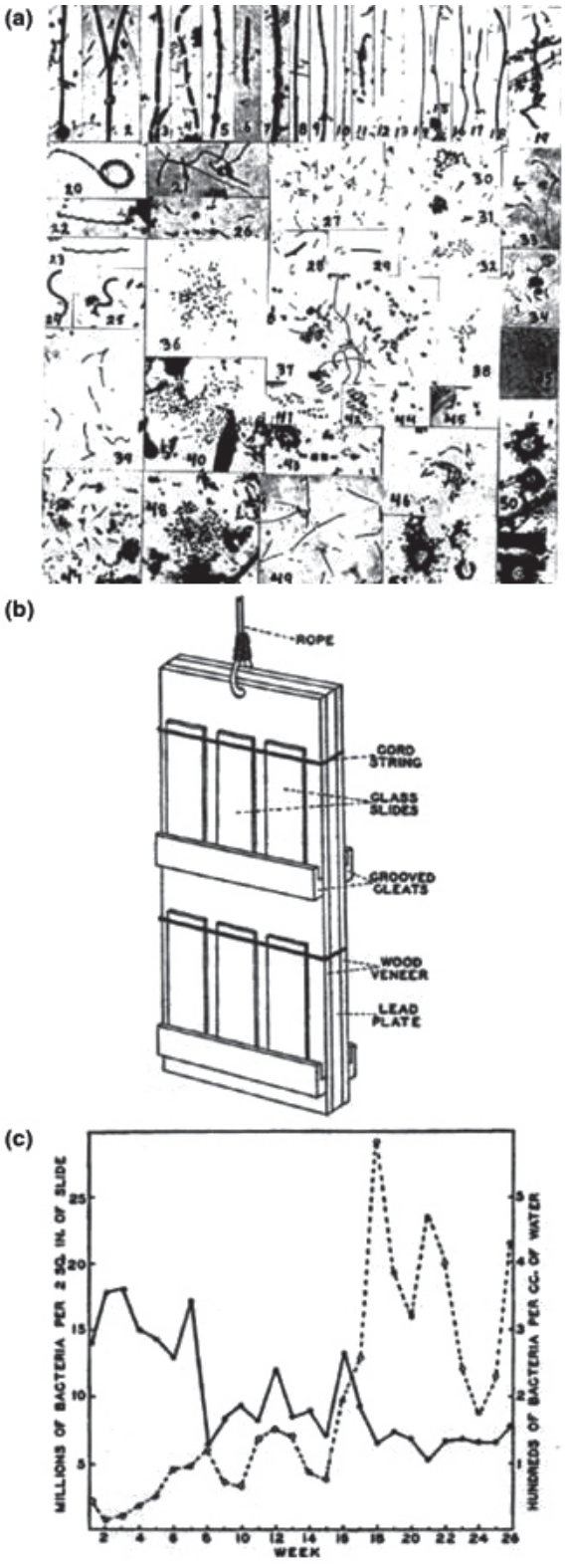

Fig. 2. (a) - Henrici's drawings of freshwater bacteria adhering to submerged microscope glass slides; **(b)** ZoBell & Allen's carrier for submerged microscope glass slides for study of salt water bacteria adhering to the slides as the first step of biofouling; **(c)** ZoBell & Allen's Fig. 2 showing the number of bacteria per cm^2 of salt water and attached to 6.25 cm^2 of the surface of the submerged slide.

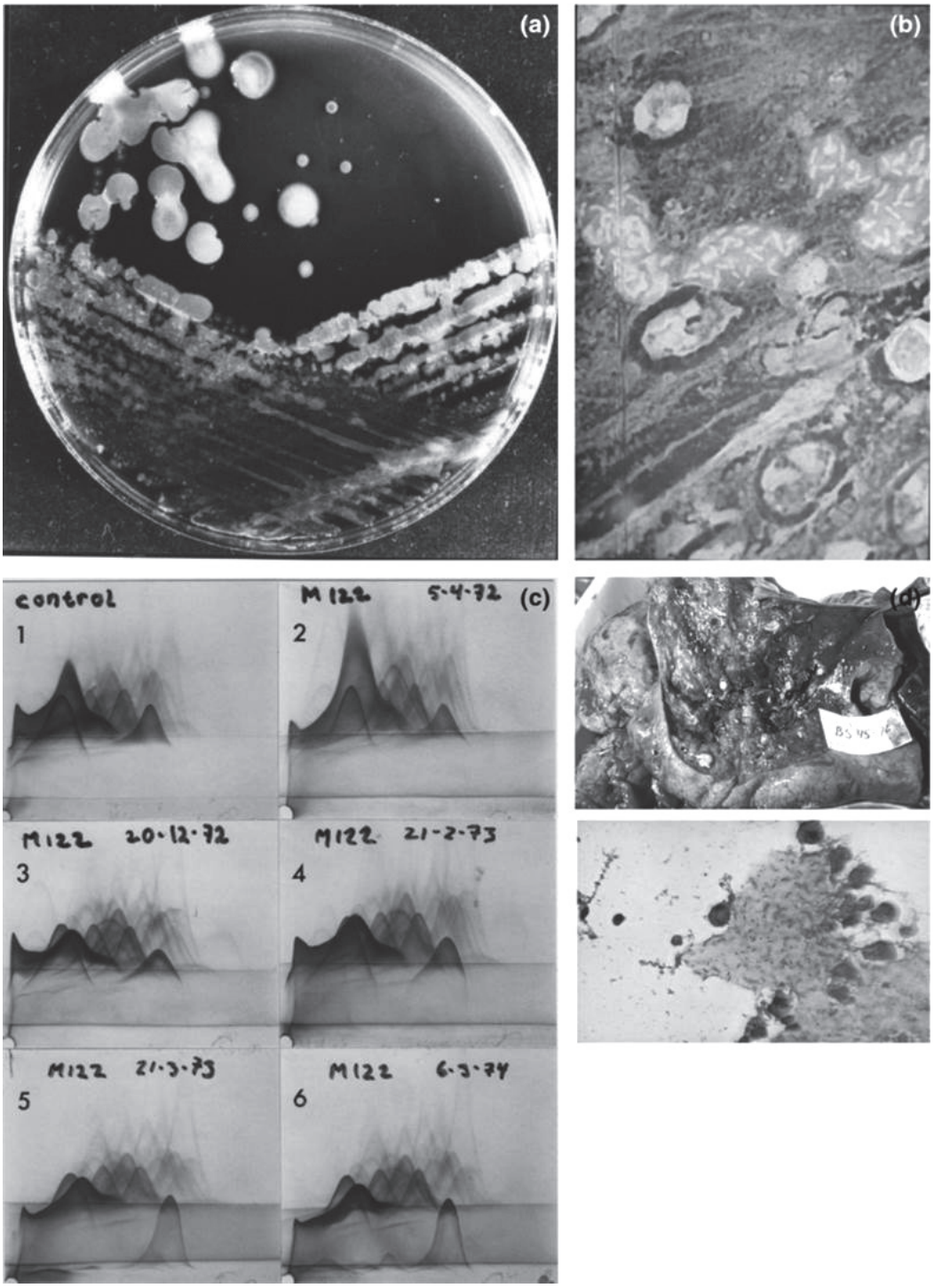

Fig. 3 - Original figures and slightly adapted legends from Høiby, 1977 (a–c). (a) Mucoid (large) and non-mucoid (small) colonies of *Pseudomonas aeruginosa* isolated from a CF patient (original Fig. 1); **(b)** Gram-stained smear of sputum from a CF patient. Mucoid *P. aeruginosa* and polymorphonuclear leukocytes are seen. Characteristically, non-mucoid and mucoid variants are seen in the same smear, the non-mucoid variants (not seen in this photograph) lacking the slime accumulations which keep the mucoid variants together in heaps. 1000× (original Fig. 2); **(c)** Serial investigation of serum from a CF patient (M122) during chronic infection with mucoid *P. aeruginosa* in the respiratory tract. Crossed immunoelectrophoresis with intermediate gel of 2 μL *(P. aeruginosa* sonicate) of St-Ag batch 3–15 against (hyperimmune rabbit) St-Ab pool 7–17 in the reference gel (20 μL/ cm^2). The intermediate gels contained: 1, saline as control; 2–6, serum from the patient taken at different times (given in the figure) before (2), and during the infection (3–6; 40 μL/cm^2). The most pronounced changes are seen from 4 to 5 (1 month; original Fig. 8); **(d)** Autopsy (upper panel) of the lungs of a CF patient who died in 1976 due to chronic *P. aeruginosa* lung infection and a large biofilm surrounded by polymorphonuclear leukocytes, which was present in the pus in the lungs (lower panel, ×1000, Gram stain).

ranging from human tooth or lung (…) 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"

Høiby met Costerton (1934-2012) at the 2nd Canadian Cystic Fibrosis (CF) Professional Conference 'Pulmonary infection in cystic fibrosis', October 15-17, 1977 in 'Alpine Inn' outside Montreal, Canada where Høiby gave a lecture 'Bacteriology and immunology of persistent Pseudomonas infection' and showed slides with mucoid and non-mucoid *P. aeruginosa* colonies from a CF patient and a biofilm in sputum from a CF patient with chronic *P. aeruginosa* lung infection (Fig. 3) and suggested that the slime (alginate) around the aggregated bacteria could protect them from being opsono-phagocytosed by the specific antibodies and the many polymorphonuclear leukocytes which were present in CF serum and sputum (Fig. 3C) [8]. Costerton gave a lecture on bacterial glycocalyx of sessile versus planktonic bacteria. During the conference, Costerton and Høiby walked for a couple of hours in the environment and discussed the significance of 'cryptic infection' as Costerton named it - he had not studied alginate at that time, but other polysaccharides in the glycocalyx - and the alginate which kept the bacteria together in 'heaps' as Høiby named it. This was the start of a very fruitful cooperation [10, 11, 12, 13, 14] and a life-long friendship between Costerton and Høiby. Costerton´s interest in CF and chronic lung infection was due to the fact that one of his children suffered from CF. Costerton's group published electron microscopy observations of *P. aeruginosa* microcolonies in a post mortem CF lung in 1980 [15] and surveys on the bacterial glycocalyx in nature and disease [16] and later he exchanged 'glycocalyx' with 'biofilms' in a new survey [17].

Costerton introduced 'biofilm' growth in medical microbiology in 1985 [18] and demonstrated the increased resistance of biofilm growing bacteria compared to planktonically growing and he subsequently pioneered the work on physiology, biochemistry etc. of biofilm growing bacteria [18]. Thereafter 'biofilm' gradually became accepted as the most adequate word for description of sessile growth *in vivo*.

Costerton was without doubt the initiator and promotor of the term biofilm in medicine which he imported from technical and environmental microbiology. His contribution in medical microbiology and infectious diseases in this respect is impressive since he was not an M.D. He paved the way for the general understanding in medicine of the significance of the concept of biofilm infection especially as regards chronic infections and foreign body infections e.g. on medical devices. His success was due to his entrepreneurship, convincing lectures and publications combined with his charming and persuasive personality and ability to listen and discuss with other people and subsequently his important position as director (1993-2004) of the large Center for Biofilm Engineering in Bozeman, Montana State University, Montana, USA.

In 2013, at the Faculty of Health and Medical Sciences at University of Copenhagen, a unique interdisciplinary biofilm research center was established, with Prof. Michael Givskov as Director. In the memory of Bill Costerton who died 12 may 2012, the center received the name Costerton Biofilm Center (https://biofilm.ku.dk/). The center aims, at the delivery of new approaches to address key challenges within medical biofilm research, to generate fundamental insights into the biology of chronic infections. The research activities include basic research in order to generate cutting edge and profound knowledge about biofilms as the basis for the development of new means of diagnosis, prevention and treatment of chronic bacterial infections with the intent of improved public health and economic gain.

A search on Web of Science using 'biofilm' as search word showed that the first biofilm publication was in 1961 by Rogovska T. and Lazareva M. in Mikrobiologiya (USSR) and described a biofilm used in water purification [19]. The first two medical biofilms reports were published in 1981 by dentists from the

University of Lund, Sweden [20, 7] in the same year when Costerton first used the term 'biofilm' in a technical microbiology report [21].

The number of biofilm publications rose slowly from 6 in 1981 to 350 in 1996 when Costerton organized the first ASM biofilm conference in Snowbird, Utah, US. The yearly number of biofilm publications continued to increase steadily and reached 6293 in 2017 (recorded August 2018) and the accumulated number was then 55.904.

Besides the description of biofilms and the understanding of their role in the pathology of a wide range of infections [22], the perception of biofilms has changed considerably in the last four decades as a consequence of the technological development and adaptation to biofilm science, including new imaging technologies, biochemical methods and molecular ecosystem biology tools [23]. It is now possible to get an overall view of the 3D biofilm structure and a detailed knowledge of the structure down to the nano-scale level [24] as well as a deeper understanding of the physiology of biofilm cells, the genotypic and phenotypic variation among the biofilm communities, as well as the biofilm metabolome [25], proteome[26] and *in vitro* and *in vivo* transcriptome [27].

In conclusion, the observation of aggregated microbes adhering to surfaces or located in tissues or secretions and surrounded by a self-produced matrix is as old as microbiology, but the concept of biofilm infection and their importance in medicine especially with respect to chronic infections is only 40 years old. Despite major achievements leading to a deep understanding of the biofilm biology, biofilm infections still represent a major diagnostic and therapeutic problem worldwide as clinical microbiologists have not yet developed methods which are suitable for routine examinations and for reports to the clinicians of the properties of biofilm growing microbes during daily diagnostic work on samples from patients and there is only consensus on treatment of a few biofilm infections (ESCMID guidelines) [28].

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