# Bacterial biofilms, antibiotic resistance and healthcare-associated infections: a dangerous connection.

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In 2012, were estimated 6.7 million cases of healthcare-associated infections (HAI) either in long-term care facilities or acute-care hospitals from which result 37,000 deaths configuring a serious public health problem [1].
The etiological agents are diverse and often resistant to antimicrobial agents. One of the mechanisms responsible for the emergence of drug resistance is biofilm assembly. Biofilms are defined as thin layers of microorganisms adhering to the surface of a structure, which may be organic or inorganic, together with the polymers that they secrete [2]. They are dynamic structures which experience different stages of organization with the ageing and are linked to an increase in bacterial resistance to host defense mechanisms, antibiotics, sterilization procedures other than autoclaving, persistence in water distribution systems and other surfaces. The understanding of bacteria organization within the biofilm and the identification of differences between planktonic and sessile forms of bacteria will be a step forward to fight HAIs.
Bacterial isolates were grown in adequate medium. Antibiotic susceptibility was evaluated by broth microdilution method and interpreted according to NCCLS guidelines. A similar assay was performed to evaluate biofilm susceptibility to antibiotics. Bacteria ability to assemble biofilms was assayed by the microtiter-plate test [3] being tested in both abiotic (materials present in healthcare units) and biotic (Hella cells) surfaces. The biofilm structure was assessed by scanning electron microscopy (SEM) in either backscattered electron diffraction or secondary electrons mode.
The kinetic of biofilm assembly depends on bacteria growth rate, incubation temperature and medium. Furthermore, the SEM analysis of planktonic and sessile forms of the same bacteria allowed the identification of structural differences which may be involved in virulence (Fig. 1). Bacteria ability to assemble biofilms seems to be independent of the abiotic structure (Fig.2). The same is not observed in biotic surfaces. This fact suggests that biofilm assembly *in vivo* is dependent of bacteria tropism. The minimum inhibitory concentration (MIC) determine for bacteria organized in biofilms is higher than for their planktonic forms. The increase ranges from 2 to 200 folds and is proportional to the ability of bacteria to assemble biofilms.
Further studies will be conducted in order to prevent biofilm assembly within healthcare units which will result in a decrease of HAI and emergence of antibiotic resistant bacteria.
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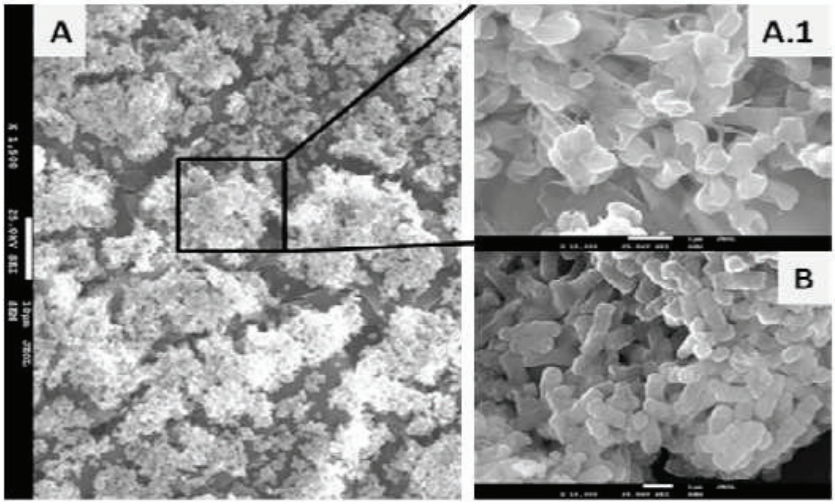


Fig. 1 – Bacteria structure: Planktonic *versus* biofilm.
A panoramic view of a 12h old biofilm of *K.pneumoniae* 703;O:1 obtained by scanning electron microscopy in secondary electrons mode is shown in figure 1.A. The structura diferences between bactéria organized in biofilm (1.A.1) and planktonic bactéria (1.B) were highlighted by this technique.

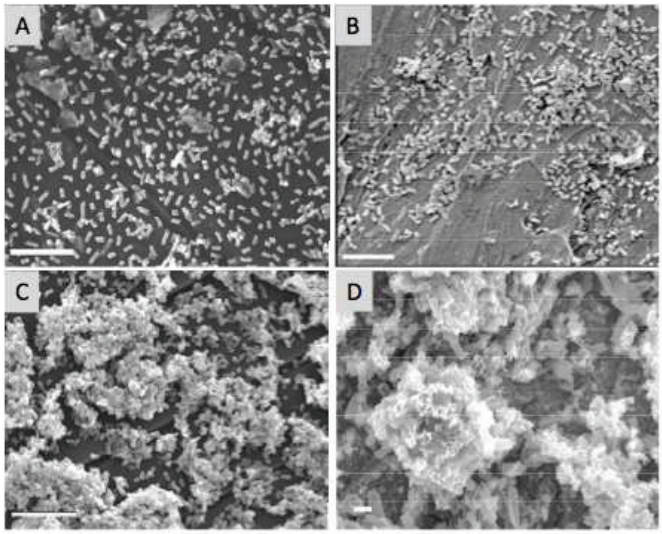


Fig. 2 – Bacteris biofilm assembler on different abiotic surfaces presente in healthcare units.
Twelve hours old biofilms assembled either on silicone (mimicking edical instruments coating e.g. cateter) or metal (mimicking water system pipes) of two different strains of *K. Pneumoniae* are shown. The bility of *K.pneumoniae* 2948 to assembler biofilm either on silicon (A) or on metal (B) is reduced in comparison to *K.pneumoniae* 703;O:1 for both materials. The image were acquired by SEM (bars=1μm).