Impact of nutritional conditions on colony morphology variants isolated from *P. aeruginosa* and *S. aureus* biofilms

Sousa, A.M., Machado I., Pereira M. O.
IBB – Institute for Biotechnology and Bioengineering, Centre of Biological Engineering, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal

In natural habitats, microorganisms are challenged all the time due to stress conditions imposed by the surrounding environment. To adapt to these environmental changes, bacteria alter their physiological and genetic traits. This adaptive behavior may be achieved by phenotype switching. This process consists in a reversible switch of phenotypes, as a mechanism ON/OFF, which occurs at high frequencies than spontaneous mutations. Colony morphology variation is the macroscopic feature of the phenotypic switching. Colony variation may have serious impact on bacterial virulence and antimicrobial resistance potentiating its ability to cause disease. Some colony variants are strongly associated to antibiotic resistance due to their presence in chronic infections despite antibiotic therapy. In cystic fibrosis, the switch of *P. aeruginosa* from non-mucoid to mucoid morphotype, which overproduce alginate, is a crucial stage to the establishment of this recalcitrant disease. Small colony variants (SCV) are other well-known resistant morphotype. These variants exhibited small size because its slow growth rate, pigmentation, haemolysis, reduced range of carbohydrate utilization and higher resistance to aminoglycosides antibiotics and cell-wall inhibitors.

In vitro in inhibitory activity of vancomycin, daptomycin, linezolid and tigecycline against methicillin resistant *Staphylococcus aureus* (MRSA) isolated from clinical samples.

Hernández, M. Lecuona Fernández

**OBJECTIVE**
To compare the *in vitro* inhibitory activity of vancomycin, daptomycin, linezolid and tigecycline against methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from clinical samples.

**MATERIAL AND METHODS**
All the MRSA isolates collected at Hospital Universitario de Canarias (Tenerife, Spain) during the period 2009-2010 were included in the study. Only one isolate per patient episode was considered. The identification and susceptibility testing were performed by VITEK2 (bioMerieux ®, France). Methicillin resistance was confirmed by using the MRSA-screen test (Denka Seiken Co., Japan) to detect the PBP2a protein. In addition, an in-house real-time PCR was carried out to detect the mecA gene. MICs were determined by the broth microdilution method according to CLSI recommendations using Cation-Adjusted Mueller-Hinton II Broth (Becton Dickinson, USA). For daptomycin susceptibility testing, the broth was supplemented to reach a Ca²⁺ final concentration of 50 µg/ml. Fresh medium (<15 hours) was used for tigecycline susceptibility testing.

**RESULTS**
Data showed that *P. aeruginosa* and *S. aureus* colony morphotypes are strongly influenced by the plating medium used. The main differences observed were the size, texture and form of colonies. The largest colonies derived from biofilms. This study allows concluding that, in contrast to fungi, bacterial colony appearance is influenced by the nutritional conditions of the solid media used to spread the cells. This evidence should be taking into account when important phenomena as phenotypic switching are going to be studied. The data obtained with this preliminary work may question the classification of colony morphotypes used until now.

**CONCLUSION**
The data show that all the MRSA isolates included were susceptible to the four antimicrobial agents tested in vitro. However, the MIC₅₀ is only 1, 1 and 2 fold dilutions lower than the susceptibility breakpoint for vancomycin, daptomycin and tigecycline, respectively. Regarding linezolid, the MIC₉₀ was equal to the susceptibility breakpoint.

**KEYWORDS:** MRSA, vancomycin, daptomycin, linezolid, tigecycline.