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.

It has been growing the number of studies related with phenotypic switching and colony morphology characterization. However, normally each study reports the use of different solid growth media which makes the comparison between studies inaccurate. In order to clarify the role of nutritional conditions on bacterial colony morphologies and on its populational diversity, *P. aeruginosa* and *S. aureus* planktonic and biofilm-growing cells were spread onto the most common solid laboratory media (TSA, MHA, LB agar, MacConkey agar and Columbia horse blood agar). Additionally, the reproducibility of each medium was also inspected.

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 were detected in TSA, MHA and LB agar. Colonies grown on MHA and LB agar were very similar possibly due to their identical nutritional composition. All the solid media tested showed reproducibility between assays except the Columbia horse blood agar which exhibited some inconsistency probably due to the presence of blood in its composition. Amongst the solid media tested, for planktonic and biofilm cultures, TSA gave rise to higher number of colony variants. Phenotype diversity seems to be more influenced by nutritional factors when bacteria 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.

Keywords Phenotypic switching; colony morphology, nutritional conditions, solid growth media

Acknowledgements: The financial support from IBB-CEB and Fundação para a Ciência e Tecnologia (FCT) and European Community fund FEDER, trough Program COMPETE, in the ambit of the Project PTDC/SAUESA/6460912006 /FCOMP-01-0124-FEDER-007480 and Idalina Machado PhD Grant (SFRH/BD/31065/2006 and are gratefully acknowledged.

In vitro inhibitory activity of vancomycin, daptomycin, linezolid and tigecycline against methicillin resistant *Staphylococcus aureus*

B. Gómez-Alonso, Z. Díaz Cuevas, A. Tenorio-Abreu, A. Alaoui Sosse, M. Hernández Porto, B. Castro Hernández, M. Lecuona Fernández

OBJETIVE

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 Ca^{2+} final concentration of 50 ug/ml. Fresh medium (<15 hours) was used for tigecycline susceptibility testing. *S. aureus* ATCC 29213 and *E. faecalis* ATCC 29212 were included as quality control strains. Susceptibility interpretations were performed according to the CLSI (2010) criteria, when available. A tigecycline susceptibility breakpoint of 0,5 µg/mL was used in accordance with Guide to Antimicrobial Therapy (J. Mensa et al, 2010),

RESULTS

During the study period, a total of 88 MRSA isolates (including 17 from Intensive Care Unit patients) were collected from 54 exudates, 17 blood cultures, 13 respiratory samples and 6 urine specimens. Regarding patients, the male-female ratio was 55-33 and the average age was 67 years (SD = ± 17.33), ranging from 21 to 92 years. Comparative data for the inhibitory activity and interpretation of susceptibility to the antimicrobial agents are shown in the following table:

Antimicrobial agents	MIC (µg/mL)			Susceptibility (%)
	Range	50%	90%	
Vancomycin	0,125 - 2	0,5	1	100
Daptomycin	0,125 - 1	0,5	0,5	100
Linezolid	1 - 4	2	4	100
Tigecycline	<0,0016 - 0,5	0,0625	0,125	100

CONCLUSION

The data show that all the MRSA isolates included were susceptible to the four antimicrobial agents tested in vitro. However, the MIC_{90} is only 1, 1 and 2 fold dilutions lower than the susceptibility breakpoint for vancomycin, daptomycin and tigecycline, respectively. Regarding linezolid, the MIC_{90} was equal to the susceptibility breakpoint.

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