Identifying genetic determinants of persistent *Staphylococcus aureus* during bacteremia and lower respiratory tract infections

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OBJECTIVE

To identify genetic determinants or patterns from *Staphylococcus aureus* that may be related to its persistence in blood of patients with bacteraemia and in tracheal aspirates of patients undergoing mechanical ventilation despite a treatment adjusted to susceptibility test results.

MATERIAL AND METHODS

Study populations. Two cohorts were analyzed: patients with *S.aureus* bacteraemia and patients admitted at ICU and under mechanical ventilation with diagnosis of bronchial colonization, tracheobronchitis and pneumonia from whom *S. aureus* was isolated in endotracheal aspirate sample (ETA). Clinical and epidemiological variables were recorded for all patients. **Persistence** was considered in cases of repeated isolation from blood cultures or ETA, respectively, after 72 h of therapy adjusted to susceptibility test results. Based on the recovery of *S.aureus* after 72h, both cohorts were split in "persistently" and "transiently" infected subgroups.

Strain's phenotypical and genotypical characterization. Clinical strains (one strain/patient) were phenotypically characterized by conventional identification methods. Strains were frozen and, subsequently, genotypically characterized with a commercial DNA microarray (*S. aureus* Genotyping, Alere Technologies, Germany). DNA microarray data include the clonal complex affiliation, and detects among others, the presence of **specific resistance and virulence genes** and their allelic variants (Figure 1). Presence of a specific gene can be categorized as positive, negative or ambiguous.

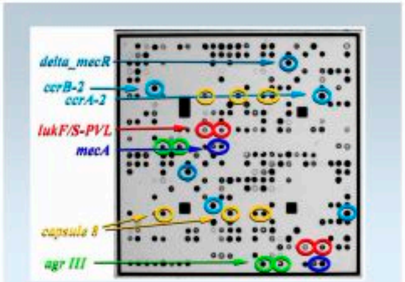

Figure 1. Genotypical characterization by means of DNA microarray.

Genome wide association studies. Genome-wide association tests were performed using a recently described statistical tool for mapping the genes that influence bacterial phenotypes of interest (http://www.danielwilson.me.uk/virulogenomics.html). The Bonferroni method was used to correct for multiple testing. Manhattan plots were used to summarize the results representing each individual gene and its association to the phenotype of interest (persistence). The bacterial population structure was taken into account when testing for an association between the phenotype of interest and the genetic variants selected.

RESULTS

- A total of 81 respiratory strains were analyzed and all DNA microarray results were valid .
- There were 43 cases classified as "transient" and 38 as "persistent".
- Table 1 shows the distribution of cases according to the study group considered and according to cloxacillin resistance. There were no statistical differences between the phenotype of interest and the study group considered nor the cloxacillin resistance pattern.
- Manhattan plots (Figure 2) show that no genes appear to be strongly associated with persistent isolation, even after correcting for population structure .

Table 1. Distribution of study group and cloxacillin resistance pattern according to the phenotype of interest for respiratory strains.

Study group	Transient (%)	Persistent (%)
Colonization (n=26)	14 (32.5)	12 (31.6)
Tracheobronchitis (n=32)	18 (41.9)	14 (36.8)
Aspiration pneumonia (n=1)	1 (2.3)	-
Pneumonia (n=22)	10 (23.3)	12 (31.6)
Total (n= 81)	43 (53)	38 (47)
Cloxacillin resistance		
MRSA (n=14)	6 (42.9)	8 (57.1)
MSSA (n=67)	37 (55.2)	30 (44.8)

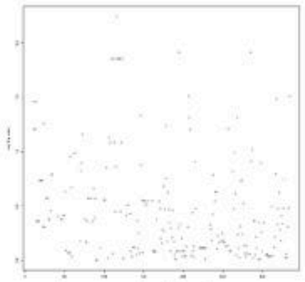

Figure 2. Manhattan plot for the respiratory strains. In the X axis are represented individually all the genes in arbitrary order, and in the Y axis the negative log base 10 p-value. Cut off point at 5% after Bonferroni correction is 3.210.

- A total of 86 bacteraemia strains were included, and 9 were considered "persistent" and 77 were considered "transient" (Table 2).
- The gene *ermC* showed a substantially stronger association with persistent bacteremia than other genes, whether or not the analysis accounts for population structure (Figure 3). However, this potential association has to be cautiously interpreted.

Table 2. Distribution of bacteremia strains according to the phenotype of interest and cloxacillin resistance.

Study group	Positive BC after 72h	Negative BC after 72h
Bacteremia (n= 86)	9 (10.4)	77 (89.6)
Cloxacillin resistance		
MRSA (n=22)	4 (18.1)	18 (81.9)
MSSA (n=64)	5 (7.8)	59 (92.2)

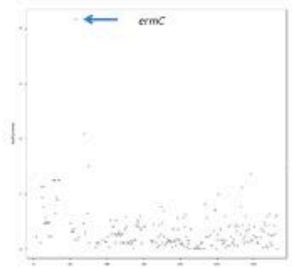

Figure 3. Manhattan plot for the bacteremia strains. Cut off point at 5% after Bonferroni correction is 3.215.

- Overall results show that no significant association was found for any gene in the two study populations regarding persistence confirming our previous results obtained by conventional univariate and multivariate statistical analyses

CONCLUSIONS

- Genome wide association studies are useful to analyse complex databases combined with multiplex genotyping, e.g., microarrays or next generation sequencing.
- No significant correlations were found between bacterial genetic variants and persistence of *S.aureus* suggesting that alternative factors such as host factors might be related. Another aspect to be considered is the role of bacterial gene expression regulation, protein translation and transport.

Project funded by Sociedad Española de Cirugia Torácica (SEPAR) P054/2011 and ISCIII FIS PI13/01418; PI: Cristina Prat (crisprat2010@gmail.com)