SMART AND FAST TOOL FOR PATHOGENS DETECTION IN SEPSIS

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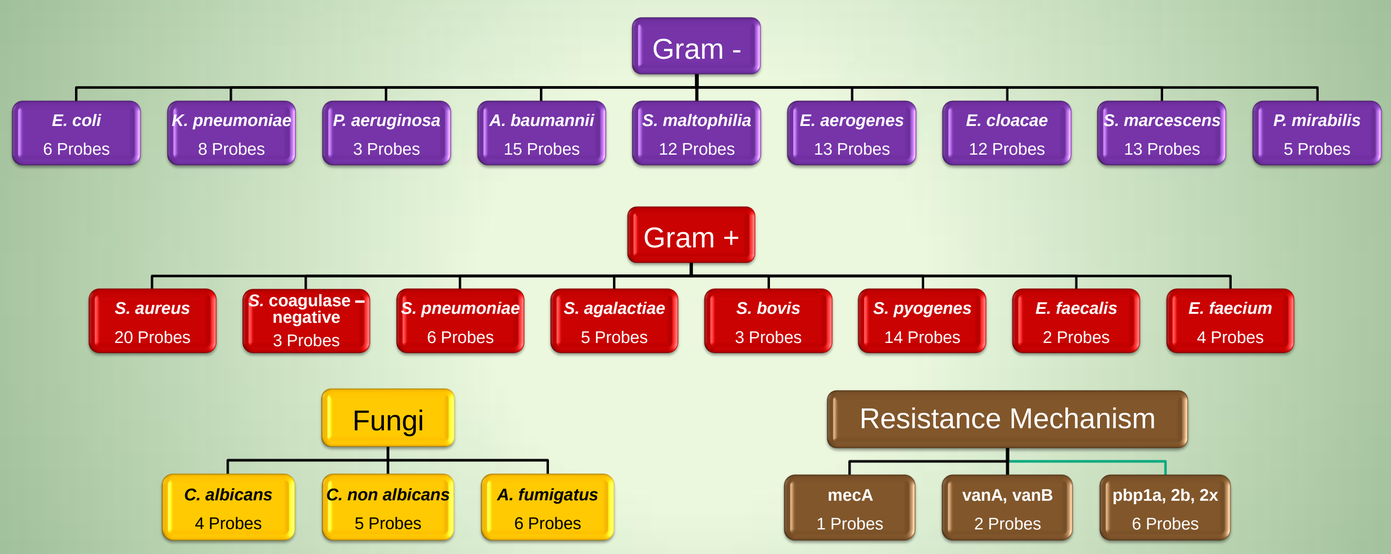
INTRODUCTION

Infections by nosocomial pathogenic microorganisms is increasingly becoming a major concern for the hospital patients. Microarray technology offers several advantages in comparison to conventional microbiological culture-based techniques and other molecular methods such as the possibility of parallel, specific and rapid detection of many different organisms in a single assay. Molecular Stamping is developing a DNA microarray-based platform for microorganisms genotyping and detection of major antimicrobial resistance genes of clinically relevant pathogens.

MATERIALS AND METHODS

The sequence of 21 complete genomes were used in order to select capture probes that provide a high discrimination capacity among different pathogens. With the aim to construct a user-friendly and cost effective diagnostic tool we restricted the size of the probe set to a maximum of 20 capture probes per microorganism. Figure 1 details the number of capture probes assigned to each pathogen.

Fig.1



SEPSI-1H DNA microarray was produced using Molecular Stamping proprietary technology. DNA was extracted as described in L.Collini *et al.* NEWMICRO poster and labeled with ULS arrayCGH Labeling Kit (Kreatech Diagnostics).

Hybridization was carried out using proprietary Molecular Stamping procedure that takes **less than two hours**. The slides were scanned with a ProScanArray™ HT Microarray (PerkinElmer). Scan images were processed by applying the GenePix software to obtain raw intensities.

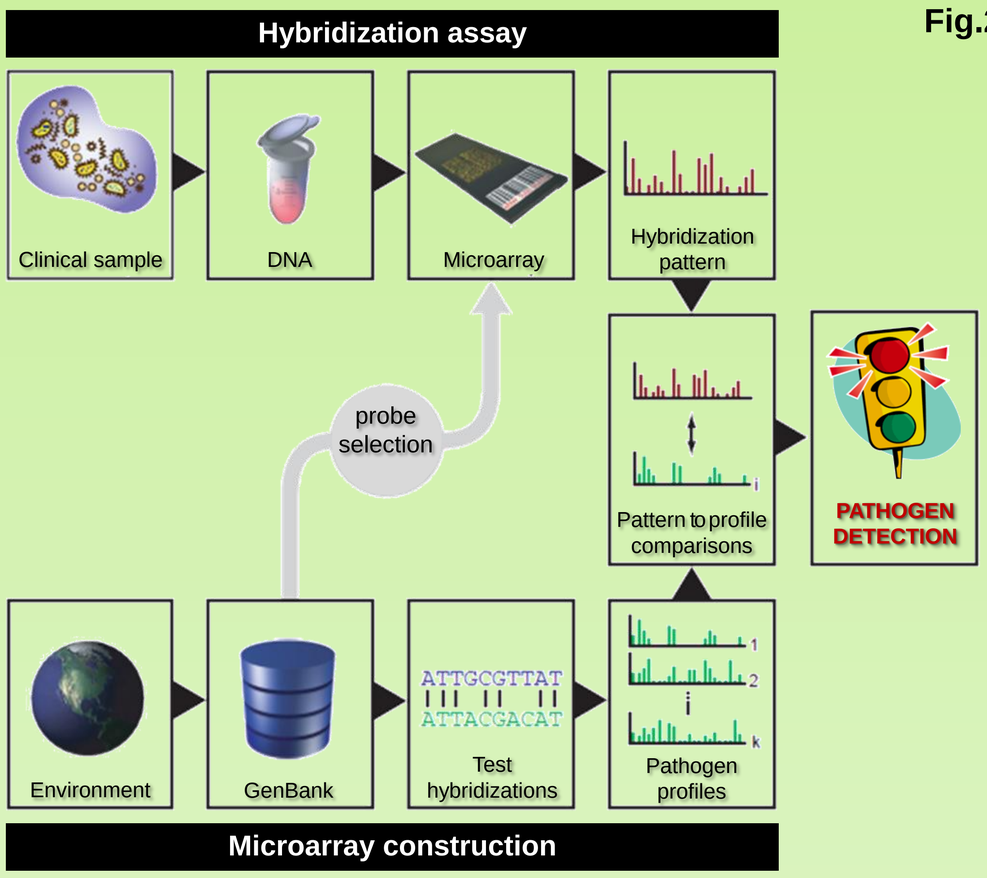
CONCLUSIONS

Notwithstanding the availability of effective antibiotics, the mortality rates associated with sepsis range between 20%-50%. An appropriate antimicrobial administration leading to a successful treatment depends on early and accurate pathogen diagnosis. Faster diagnosis of infections would reduce morbidity and mortality through the earlier implementation of appropriate antimicrobial treatment. Molecular Stamping DNA-based microarray diagnostic platform is a promising tool for a rapid and broad spectrum identification of microorganisms involved in sepsis.

RESULTS

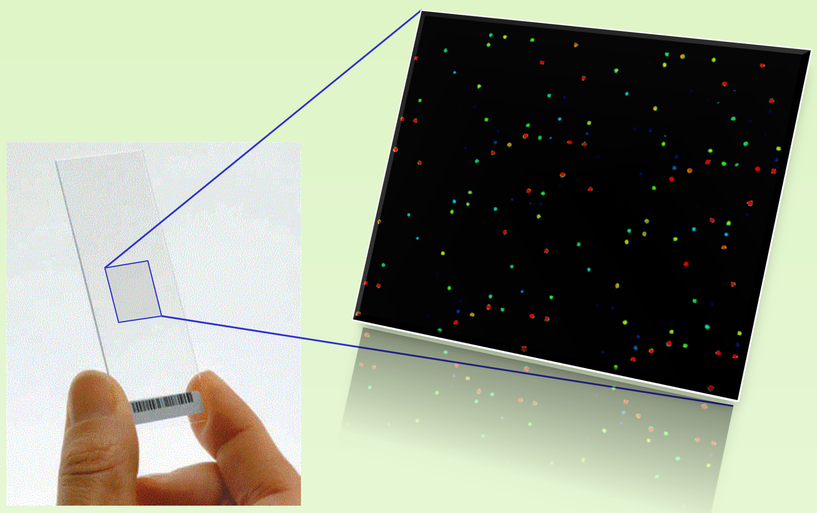
Samples of genomic DNA (gDNA) extracted from representative strains of various pathogens were hybridized on the microarray in order to determine its classification performance. Test hybridizations with 2 different clinical samples (sample 6 and 7, see in L. Collini *et al.* NEWMICRO poster) were set up in addition to pure culture tests in order to evaluate the performance of the microarray on real hospital samples. The labeled gDNAs were hybridized on SEPSI-1H microarray and hybridization patterns were analyzed to identify particular pathogens that are present in the samples (Figure 2).

Fig.2



Thanks to this identification strategy for detecting microorganisms we were able to identify sample 6 as *Staphylococcus aureus.* The particular pattern of sample 7 indicates that it also belongs to *Staphylococcus* genus. Figure 3 shows the hybridization assay of labeled sample 6 genomic DNA on the SEPSI-1H microarray. The positive signals confirm that the tested strain belongs to *Staphylococcus aureus*.

Fig.3



These first results showed a high degree of reproducibility between replicate hybridizations as well as good correlation with the results obtained by conventional microbiological analysis.
Validation in larger patient cohort and in diverse clinical settings will be an important next step towards establishing the clinical role of SEPSI-1H.

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