A multiplex platform for the detection of antibiotic resistance

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Background: Antibiotics have an effect on many different processes within bacterial cells. If the targets of antibiotics are affected by mutational events such as point mutations, the bacterium may protect itself from the biochemical mechanisms of the antibiotic and consequently become resistant. These resistances are transmitted from bacterium to bacterium by vertical and horizontal gene transfer and can thus spread rapidly in bacterial populations. If phenotypic rather than genotypic determination methods are used to determine resistance, a delayed or possibly inaccurate diagnosis may result. In the case of infection with multidrug-resistant pathogens, this could mean that patients do not receive the appropriate antibiotics for their treatment.

Methods: The goal of the study was to develop a multiplex platform for exposure and labeling of point mutations in regards to an innovative solid-phase linked microarray with reverse hybridization approach. Sub-steps of the work included the development of an internal control to verify the error-free running of the assay. An asymmetric qPCR was used to amplify ssDNA which was then used in a sequenase reaction. In this sub-step of the assay, the point mutations are labeled with specific fluorescence dyes. The plate was then read by using the well-established AID Reader System *i*Spot Spectrum.

Results: Several approaches with plasmids harboring antibiotic resistance genes such as *bla*$_{TEM}$, *bla*$_{SHV}$ and- *bla*$_{CTX-M}$, in single and multiplex approaches, using different polymerases, were tested and correctly identified. Furthermore, several positive and negative ESBL-containing samples were applied on the array.

Discussion: The microarray presented in this case study can represent a significant contribution to the optimization of the process of faster and more effective diagnosis of antibiotic resistant bacteria. It can be performed easily and gives the opportunity to be embedded in a high throughput workflow. It has the potential to be a major advantage in medical diagnostics.