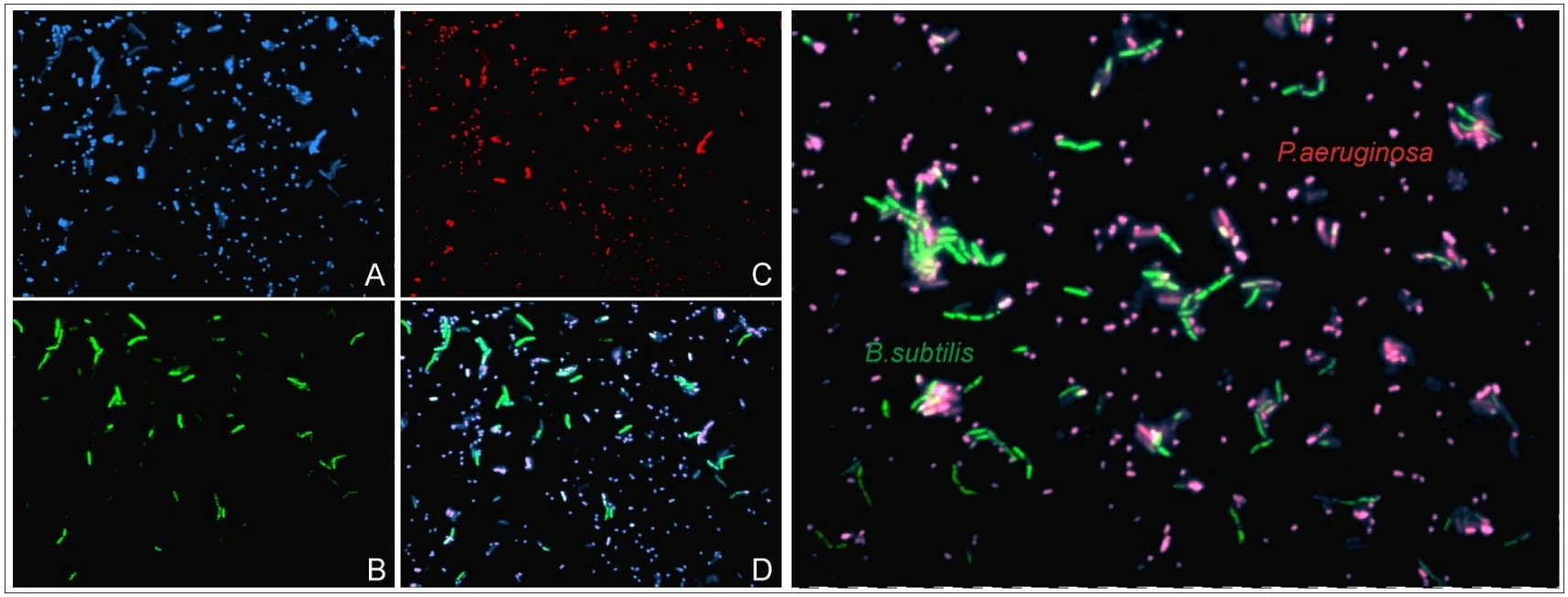
Detection and discrimination of a common, often multidrug-resistant pathogen: *P. aeruginosa*

We further validated our approach by detecting a well-known, often multidrug-resistant pathogen, *P. aeruginosa*. *P. aeruginosa* is a major cause of morbidity and mortality due to healthcare-associated infections. Since the outer membrane of *P. aeruginosa* is less permeable than of other Gram-negative bacteria, it is intrinsically resistant to many antimicrobials. This outer membrane therefore presents a potential technical challenge since our protocol depends on PNA and RCA molecules being able to easily enter bacterial cells. Based on genomic sequences of two *P. aeruginosa* strains that were available in Bacteria Genomes Database and the availability of PNAs in the laboratory, we chose three unique target sites specific for different gene regions (Table 1). . Then we examined whether we are able to reliably distinguish *P. aeruginosa* from other species using the selected signature sites. For reference species we used bacteria and signature sites previously studied while developing our approach (6, 7).

Supplementary Figure 5 shows typical results obtained with *P. aeruginosa* and *B. subtilis* when any combination of chosen sites for each bacterium was used for detection. *P. aeruginosa* and *B. subtilis* are morphologically distinguishable and nearly all cells in the mixture displayed red or green specific signals only. No signal was observed in control experiments when probes specific for *S. aureus* were applied (not shown). These data demonstrate high specificity of detection by our approach and they support expectations that our approach can be developed for simultaneous detection of various bacterial pathogens in clinical samples as long as appropriate sets of probes are applied.

Supplementary Figure 5. Detection and discrimination of a multidrug-resistant pathogen: *P. aeruginosa.* Images of mixture of *P.aeruginosa* and *B.subtilis* bacterial cells observed by fluorescent microscope in experiments performed directly in blood culture when mixture of probes popB specific for *P. aeruginosa* and yxjA specific for *B. subtilis* were applied. The fluorescent signals were acquired separately using three filter sets. Images are superposition of two/three separate images, with: **(A)** DAPI; **(B)** FITC; **(C)** CY3; **(D)** DAPI, CY3 and FITC. Signals are pseudocolored in blue for DAPI, red for CY3 and green for FITC, respectively. *Image in right*: Simultaneous detection of two bacteria in blood by the proposed approach.


A
C
B
D
P.aeruginosa
B.subtilis