P1234

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Methods of antibacterial susceptibility testing

Rapid antimicrobial susceptibility profile directly from positive blood cultures using flow cytometry

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Objectives

Early detection of antimicrobial resistance is crucial for correct patient management. It would prevent starting empiric treatment, often broad spectrum, with great impact upon antimicrobial resistance and hospital infection control. The recent use of matrix-assisted laser desorption ionization time-of-flight technology (MALDI-TOF) represented a great progress for routine laboratories allowing a quick identification of microorganisms from blood cultures but it does not provide its respective antimicrobial susceptibility phenotype. Flow cytometry represents an excellent tool for rapid evaluation of antimicrobial susceptibility. Using the correct fluorescent probe, it is possible to detect lethal or pre-lethal lesions with excellent correlation with classic method. The goal of this work was to detect resistance to the main antimicrobials directly from positive blood cultures, using BioFAST®, a flow cytometry analysis kit.

Methods

Three hundred positive blood cultures were studied corresponding to 200 Gram negative bacilli isolates (including *Enterobacteriaceae*, *Acinetobacter* spp and *Pseudomonas* spp) and 100 Gram positive cocci isolates (including *Staphylococcus* spp and *Enterococcus* spp). For routine susceptibility methods, blood cultures were sub-cultured in Colombia agar and the identification and susceptibility performed using Vitek2 cards (bioMérieux). In parallel, MALDI-TOF MS and flow cytometric susceptibility analysis was performed directly from positive blood culture. The bacterial cells were separated from blood culture, washed and incubated for 1 h with different antimicrobials and stained according to BioFast® susceptibility kit under performance evaluation by FASTinov, Porto, Portugal. For Gram negative, meropenem, piperacillin plus tazobactam, cefotaxime, ceftazidime, gentamicin and amoxacilin plus clavulanic acid were tested; for Gram positive, vancomycin, cefoxitin/oxacillin, ampicillin and gentamicin were tested. The bacterial cells were analyzed in a FASCalibur cytometer and a staining index (SI) calculated, comparing the intensity of fluorescence of treated cells and non-treated cells. The susceptibility phenotype was determined applying cut-off values. Discrepant susceptibility phenotypes were re-tested according EUCAST microdilution susceptibility test.

Results

A good correlation between flow cytometry results and Vitek2 was found. No very major errors were encountered. Discrepant results between BioFast® and Vitek2 were registered regarding cefoxitin and ampicillin for cocci and gentamicin both for cocci and bacilli. Microdilution test results were in accordance with flow cytometry analysis in all the cases.

Conclusion

In a maximum of 90 minutes time to result, it was possible to obtain a reliable susceptibility phenotype, directly from positive blood cultures, with excellent correlation with reference method. Considerable time saving was obtained, which could represent a considerable step forward on laboratorial diagnostics.

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The present technology is undergoing patent protection (W0212164547A), thus all the disclosed information is confidential until publication in the congress abstract book or presentation delivery.