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Rapid detection of Escherichia coli and Klebsiella pneumoniae and resistance markers in positive blood cultures by using MALDI BioTyper system and FilmArray blood cultures identification panel combined with the Eazyplex Superbug CRE assay

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Background: Bloodstream infections (BSIs) caused by *Escherichia coli* and *Klebsiella pneumoniae* producing extended-spectrum β-lactamases (ESBLs), and carbapenemases are associated with increased rates of treatment failure and death, and efforts should be made to ensure that appropriate therapy is initiated promptly. Consequently, rapid and accurate identification (ID) of these organisms is critical for early initiation of adequate treatment.
We implemented our laboratory's BC protocol, based on the use of the MALDI BioTyper System and FilmArray blood culture ID (BCID) panel (bioMérieux, Marcy l'Etoile, France) for direct ID of microbial pathogens from BC broths, by adding the eazyplex® SuperBug CRE assay (Amplex Diagnostics GmbH, Germany, Biolife italiana SRL, Milano Italia) when *E. coli* or *K. pneumoniae* were identified by the direct ID procedure.

Material/methods: The study was conducted between 15 January 2015 through 15 January 2016 in the clinical microbiology laboratory of the Catholic University Medical Center, Policlinico Gemelli, Rome. First, we evaluated the eazyplex® SuperBug CRE assay with BCs spiked with well-characterized gram-negative strains. Then, blood samples from adult patients with suspected BSIs were collected. Broth aliquots from each positive BC bottle were collected for standard method (Gram

staining, and culture-based method) and direct species identification of the infecting pathogens using the method above mentioned. The eazyplex® Amplex assay with the Superbug CRE panel was performed on BC broths immediately after *E. coli* or *K. pneumoniae* were identified.

Results: In preliminary testing, carried out with the 126 spiked BCs, the eazyplex® SuperBug CRE assay displayed 100% of concordance with the comparison method. The reproducibility was excellent, with no false negatives and no false positives in the replicate tests. Out of the 425 BCs we evaluated, 263 grew *E. coli* (29 from polymicrobial BCs), 148 yielded *K. pneumoniae* (30 from polymicrobial BCs) and 14 grew both *E. coli* and *K. pneumoniae.* Overall 102 isolates carried $bla_{CTX\text{-}M}$, 54 harbored bla_{KPC}, 4 both bla_{KPC} and $bla_{VIM,}$ and a single *E. coli* isolate carried bla_{VIM}. The eazyplex® SuperBug CRE correctly detected all the KPC, VIM and CTX-M genes, with no false-positives even in polymicrobial samples or in those harboring more genes. yielding a sensitivity and specificity for each target of 100% and 100%, respectively.

Conclusions: Our approach based on the combined use of MALDI BioTyper and FilmArray BCID plus the eazyplex® SuperBug CRE system appears to be a reliable, timesaving tool for routine detection of *E. coli* and *K. pneumoniae* producing CTX-M ESBLs and carbapenemases in the setting we studied, although further studies are needed to evaluate their performance in other settings.