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Rapid detection of carbapenem resistant *Klebsiella pneumoniae* directly from positive blood cultures during an outbreak in the Tuscany region in Italy

Cesira Giordano[1]*, Alessandra Vecchione[1], Melissa Menichini[1], Antonella Lupetti[2] and Simona Barnini[1]

[1]Bacteriology Unit, Azienda Ospedaliero-Universitaria Pisana, Pisa, Italy

[2]Department of Translational Research and of New Technologies in Medicine and Surgery, University of Pisa, Italy

Introduction

In an era of increasing drug resistance, rapid detection and determination of the molecular mechanisms involved in antimicrobial resistance is pivotal for successful treatment of bloodstream infections. The appropriate antimicrobial therapy is crucial to reduce the mortality rate [1] and should be started within 1 hour of sepsis suspicion [2], but empirical therapy is a challenge in those hospitals where resistance mechanisms are an ever-changing landscape [3]. Rapid microorganism identification in positive blood cultures (BCs) has been achieved using a variety of innovative techniques [4-7]. The turnaround time of results has indeed been reduced considerably in the recent years by using the Matrix Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS) for pathogen identification in samples taken directly from positive BCs [8,9].

At the Bacteriology Unit of the Pisa University Hospital (Pisa, Italy), *Escherichia coli* and *Klebsiella pneumoniae* are the prevalent species isolated from BCs [10]. While in our region, Tuscany, *E. coli* is still sensitive to most antibiotics, carbapenem resistant strains of *K. pneumoniae* have developed and spread in the community and in healthcare settings, first as *K. pneumoniae* strains harbouring the KPC carbapenemase gene (KPC-Kp), and more recently harbouring the NDM gene (NDM-Kp) [11,12]. To guide clinicians in starting the proper therapy, we employed the CarbaR molecular kit (GeneXpert® System, Cepheid, Sunnyvale, CA, USA), which has been developed for the detection of carbapenem resistance mechanisms (bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{IMP-1}, and bla_{OXA-48} genes) from rectal swabs or from bacterial colonies. In the present study, we aimed at establishing a rapid method, able to provide detection and differentiation of the most prevalent carbapenemase gene families directly from positive blood cultures containing *K. pneumoniae*. To this aim, a bacterial pellet was recovered directly from positive BC bottles and partly used for MALDI-TOF MS identification (Bruker Daltonics, Bremen, Germany) according to a previously published protocol [10]; when bacteria were identified as *K. pneumoniae*, an aliquot of the harvested pellet was used to prepare a 0.5 McFarland suspension. Twenty µl of a 1:10 dilution in distilled water were inoculated in the Xpert® Carba-R lysis buffer and vigorously vortexed for 1 minute. Next, an aliquot (1.7 ml) was transferred into the Xpert® Carba-R cartridge for detection and differentiation. Blood samples from patients admitted to the Pisa University Hospital in the period June 2016 - May 2019 were inoculated into blood culture bottles Plus Aerobic/F and Plus Anaerobic/F, or Peds Plus F (Becton Dickinson &Co, BD, Milan, Italy). In total, 101.290 BCs were collected at the Microbiology Unit and transferred to the Bactec FX Instrument (Becton Dickinson, Franklin Lakes, NJ, USA), 10.405 of which became positive. Among the positive BC bottles, 965 were for *K. pneumoniae*. For each patient, only the first positive BC bottle was processed by the method herein described: in total, 406 *K. pneumoniae* were directly identified by MALDI-TOF MS in about 30', while the molecular detection of genes involved in carbapenem resistance took 48'. Carbapenem resistance mechanisms were found in 166 (40.9%) BCs. All strains were subsequently analysed phenotypically reaching 100% agreement with the molecular method. As shown in figure 1, the proportion of KPC-Kp producer was 45% in 2016, 43% in 2017, 31% in 2018, and 25% in 2019. Both in 2016 and in 2017, two strains of *K. pneumoniae* harbouring carbapenemase VIM gene were detected; *K. pneumoniae* harbouring carbapenemase OXA-48 gene was detected

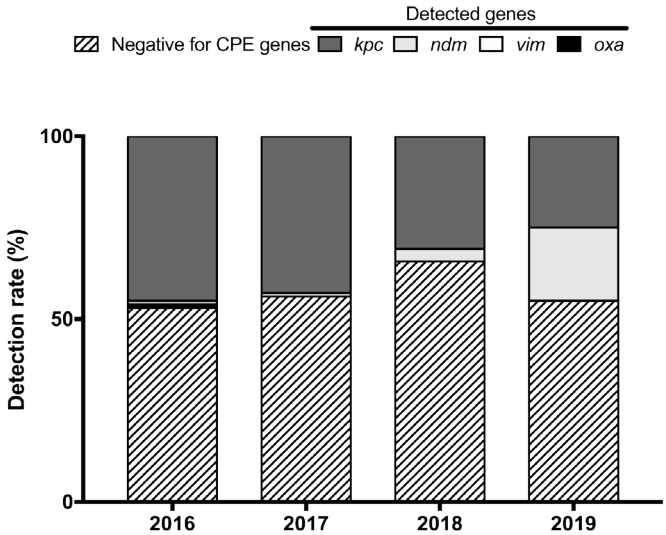

Figure 1. Blood culture trend for Carbapenamase-producing *Enterobacterales* (CPE)

Correspondence to: Cesira Giordano, Bacteriology Unit, Azienda Ospedaliero-Universitaria Pisana, Pisa, Italy, E-mail: cesira.giordano@ao-pisa.toscana.it

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only once in 2016. NDM-Kp was not detected in 2016 and 2017, whereas it was detected with a percentage of 3% in 2018, and 20% in 2019 (January-May) [12]. *K. pneumoniae* harbouring carbapenemase IMP-1 gene has not yet been detected.

In Northern Tuscany, we witnessed a progressive evolvement over time of resistance mechanisms to carbapenems, and an overpowering appearance of NDM-Kp. In July 2019, our regional health directorate issued a strict protocol for the surveillance and control of infections, in an attempt to stem the spread of the epidemic of resistant strains. In this context, a rapid method for detecting molecular mechanisms of resistance to carbapenems, directly from positive BCs, may be crucial for infection management. Indeed, patients with positive results could be promptly isolated, and differently isolated, depending on the detected mechanism of resistance. This method requires about 78' for rapid MALDI-TOF MS identification of *K. pneumoniae* and detection and differentiation of carbapenem resistance mechanisms. In conclusion, we believe that this method allows both to design effective infection control measures and to promptly administer the correct antimicrobial therapy, as it is known that the use of new drugs, eg. ceftazidime/avibactam is not effective against NDM-Kp.

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