

# Phenotypic classification of multidrug-resistant *Klebsiella* through MALDI-TOF mass spectrometry

Anna Di Taranto,<sup>1</sup> Rosella De Nittis,<sup>1</sup> Marco Fagioni,<sup>2</sup> Tiziana Rollo,<sup>1</sup> Paola Cella,<sup>1</sup> Raffaele Antonetti<sup>1</sup>

<sup>1</sup>Department of Clinical Pathology, Analysis Laboratory II, Ospedali Riuniti of Foggia University Hospital; <sup>2</sup>Bruker Daltonics s.r.l., Macerata, Italy

## Summary

**Background.** Resistance to carbapenems in *Klebsiella pneumoniae* infections could compromise therapy efficiency in hospitals. In Italy, the percentage of *K. pneumoniae* resistant to carbapenems blood-isolated is 35%.

**Materials and Methods.** Seventy-three multidrug-resistant *K. pneumoniae* clinical isolates coming from Foggia (Italy) have been phenotypically classified through matrix assisted laser desorption/ionization-time of flight mass spectrometry between February and May 2014 in order to locate an eventual infectious outbreak.

**Conclusions.** Classification of microbial isolates is essential to this purpose and starts from phenotypic methodologies to the most complex genotypic methods. Among these, mass spectrometry utilization appears to be a simple, useful and quick instrument in a clinical microbiology laboratory.

promises the therapy efficiency in the hospital environment and leads to a major degree of mortality if compared to sensitive bacteria (5). In Italy, which is the most affected State in Europe after Greece and Cyprus, the percentage of *K. pneumoniae* resistant to carbapenems blood-isolated was 35% in 2013 according to European Antimicrobial Resistance Surveillance Network (2). After locating patients who are affected or colonized by these pathogenic agents, it is essential to execute efficient inspections (1) and to set up epidemiological investigations to determine infections sources and their diffusion (4), in order to contrast the hospital carbapenems resistance increase (3). For this purpose, the phenotypic classification of clinical isolates of multidrug-resistant *K. pneumoniae* through matrix assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS), a simple and low-cost methodology compared to genotypic investigation, plays a key role.

## Introduction

Resistance to carbapenems, growing in severe Enterobacteria infections and especially in *Klebsiella pneumoniae* infections, com-

## Materials and Methods

All the multidrug-resistant (MDR) *K. pneumoniae* clinical isolates coming from wards of Azienda Ospedaliera Universitaria OO.RR of Foggia (Italy) have been phenotypically classified through MALDI-TOF MS in the period between February and May 2014, in order to locate an eventual infectious outbreak and consequently strengthen the adoption of appropriate countermeasures of infection control.

Seventy-three clinical isolates of *K. pneumoniae* have been analyzed in the period between February and May 2014, and using the BD Phoenix™ (Becton Dickinson, BD Diagnostic Systems, Sparks, MD, USA) identification system and antibiogram, they showed resistance to several carbapenems. Patients were 38 male (52%) and 35 female with an average age of 70 years; they came from Intensive Care Unit (36%), Internal Medicine (23%), Physiatry (19%), Nephrology (18%), Surgery (2%), Hematology (2%) Departments; clinical items were urines (66%), blood (19%), washing bronchial (12%) and pus (3%). The 14 blood samples, coming from Intensive Care unit in the 85% of cases, have been further analyzed with GeneXpert™ Carba-R assay (Cepheid, Sunnyvale, CA, USA), that found in all of them the KPC sequence. The isolates, after defrosting and passage on Agar blood Columbia (Becton Dickinson) and extracted with standard procedure, have been analyzed with MALDI-TOF MS Microflex™ LT (Bruker Daltonics, Bremen, Germany), reiterating 16 times for each sample to exclude casual effects. Spectra have been analyzed with ClinProt Tools 3.0 (Bruker Daltonics) software with the purpose of building a resuming dendrogram and for a statistical analysis of clusters based on principal component analysis (PCA) variance.

Correspondence: Anna Di Taranto, Dipartimento di Patologia Clinica, Laboratorio Analisi, Azienda Mista Ospedaliero-Universitaria OO.RR Foggia, Viale Luigi Pinto 1, Foggia, Italy.  
Tel.: +39.800.102.171.  
E-mail: aditaranto@ospedaliriunitifoggia.it

**Key words:** *Klebsiella pneumoniae*; carbapenems; matrix assisted laser desorption/ionization-time of flight mass spectrometry.

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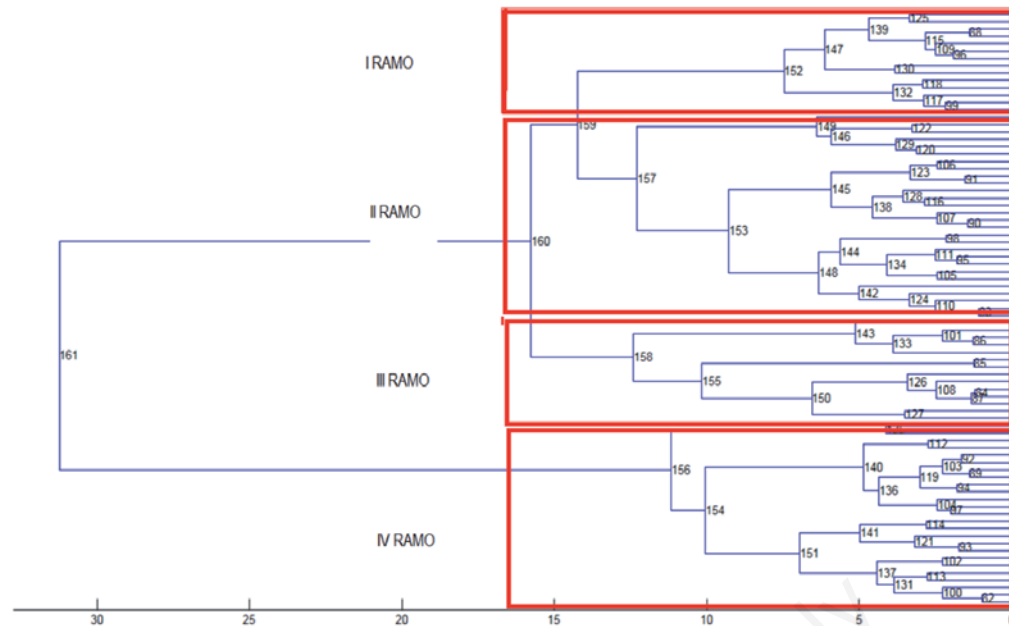


Figure 1. The dendrogram shows four distinct groups presenting a high similarity.

## Results

The dendrogram (Figure 1) shows four distinct groups presenting a high similarity, denoted with numbers from I to IV; in Physiatri and Nephrology, where all samples were urines, there is not a unique group prevalence. In the medical wards, where samples were 65% urines, stem II prevails in February and stem III in April and May. Even in Intensive Care units, where samples were 39% coming from blood, 34% LAB, 19% urines and 8% exuded, from February to April group II prevails (35%), while in May group III does (27%). This trend is confirmed in every kind of sample, particularly for blood. Statistical analysis of PCA (Figure 2) highlighted the presence of two distinct clusters (green

and blue ones) in Intensive Care 1, from which the major number of logs were coming, and consequently a possible infectious outbreak.

## Discussion and Conclusions

Classification of microbial isolates, essential to demonstrate a possible infectious outbreak, starts from phenotypic methodologies to reach the most complex genotypic methods. Among phenotypic methods, recently, mass spectrometry utilization, analyzing proteic spectra of isolates, appears to be a simple, useful and quick instrument for MDR stems diffusion prevention in a clinical microbiology laboratory. Such technique allowed to evaluate if in a given ward, *K. pneumoniae* MDR isolates did or did not belong to a unique circulating stem and consequently to foresee the need to strengthen prevention protocols of infections due to clinical support operations.

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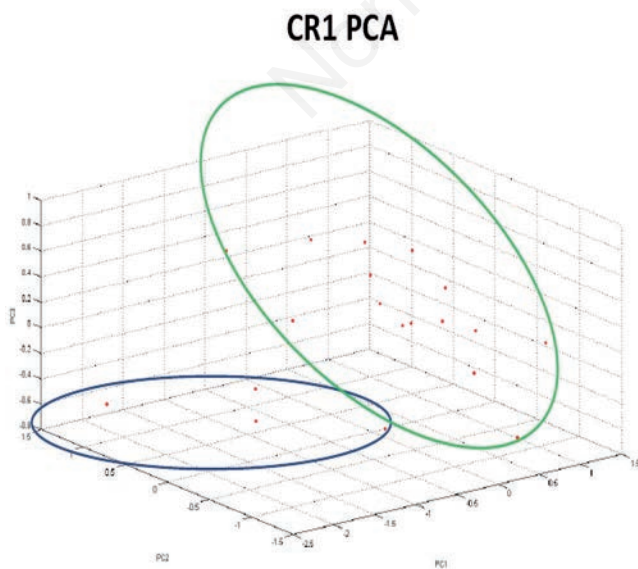


Figure 2. Statistical analysis of PCA highlights the presence of two distinct clusters (green and blue ones) in Intensive Care 1.