

# Multifocal diffusion of a KPC-3 producing ST512 *K. pneumoniae* clone in Northern Italy

Roberta Migliavacca<sup>1</sup>, Elisabetta Nucleo<sup>1</sup>, Sara Asticcioli<sup>1</sup>, Erminia Casari<sup>2</sup>,  
Silvia Bracco<sup>3</sup>, Maria C. Sironi<sup>4</sup>

<sup>1</sup>Department of Clinical Surgical Diagnostic and Paediatric Sciences, Microbiology Section, University of Pavia, Italy;

<sup>2</sup>Analysis Laboratory, Microbiology Section, IRCCS Humanitas Clinical Institute, Milan, Italy;

<sup>3</sup>Laboratory of Microbiology, A. Manzoni Hospital, Lecco, Italy;

<sup>4</sup>Laboratory of Microbiology, Niguarda Ca' Granda Hospital, Milan, Italy

## SUMMARY

Sequence Type 258 (ST258) together with its allelic single- and double-locus variants have mostly been associated with the dissemination of KPC-producing *Klebsiella pneumoniae* in Europe. A total of 56 nonreplicate *K. pneumoniae* isolates with decreased carbapenem-susceptibility, collected at 7 different hospitals located in Northern Italy were investigated for the occurrence of *bla*KPC-type genes. PCR and sequencing results highlighted the presence of *bla*KPC-2 or *bla*KPC-3 determinants in 10/56 and 5/56 cases respectively. Here we describe the intra- and inter-hospital spread in Northern Italy of a *K. pneumoniae* ST512 clone harboring the *bla*KPC-3 gene.

**KEY WORDS:** *K. pneumoniae*, KPC-3, ST512 inter-hospital spread.

Received September 14, 2012

Accepted November 06, 2012

KPC-type carbapenemases are globally spread  $\beta$ -lactamases of Ambler class A, with KPC-2 and KPC-3 variants currently being the most widespread in *Klebsiella pneumoniae* in Europe. Although expansion of both clones and plasmids seems to have fuelled the recent pandemic dissemination of *bla*KPC-encoding genes in clinical isolates, a single multilocus Sequence Type 258 (ST258) genetic background has mostly been associated with the emergence and dissemination of KPC-producers in Europe (Cantón R *et al.*, 2012).

A rapid identification of this clone along with various allelic single-locus (ST379, ST418, ST512, ST554, ST744) and double-locus (ST650, ST683, ST745) variants is of great importance for tracking further dissemination of KPCs in Italy (Giani T. *et al.*, 2009).

### Corresponding author

Dott. Roberta Migliavacca  
Department of Clinical Surgical Diagnostic  
and Paediatric Sciences  
Section of Microbiology  
University of Pavia  
Via Brambilla, 74 - 27100, Pavia, Italy  
E-mail: r.miglia@unipv.it

The aim of the study was to investigate the phenotypic/molecular features and clonal relatedness of *K. pneumoniae* isolates with decreased susceptibility collected from different Italian hospitals.

During the period June 2009-May 2011, 56 non-replicate *K. pneumoniae* clinical isolates previously identified and screened for carbapenem-susceptibility at the Microbiology Laboratories of 7 different hospitals in Northern Italy were sent to our reference Laboratory (University of Pavia). Species identification and antimicrobial susceptibilities were confirmed by Microscan System (Siemens) NBC46 panels. The strains were obtained mainly from urine (47%), blood (29%) and lower respiratory tract (11%). The samples were collected mostly from intensive care units (39%) and medical wards (27%). Carbapenemases production was initially detected in 15/56 (26,78%) cases using Modified Hodge Test; synergistic activity between ertapenem and phenyl-boronic acid confirmed the expression of a Class A enzyme in all the above strains.

Analytical IEF of crude extracts of the 15 carbapenemase-producing strains showed a pI 6.7  $\beta$ -lactamase band, able to hydrolyze imipenem (1



FIGURE 1 - KPC-3 producing ST512 *K. pneumoniae* strains: dendrogram and similarity matrix. Isolates were part of an unique clone with a similarity >98% in the DiversiLab system.

mg/L) by a substrate overlaying procedure; these isolates were positive for the presence of *bla*KPC-like genes by PCR, performed as previously described (Samuelsen Ø *et al.*, 2009).

Amplification products from PCR-positive isolates were sequenced using automated system 3700 (Applied Biosystem). Sequences were identified by BLAST program NCBI web site as *bla*KPC-2 and *bla*KPC-3 genes in 10/15 and 5/15 cases, respectively.

All KPC-producing isolates were multidrug-resistant, showing resistance to amikacin, levofloxacin, ciprofloxacin and tobramycin; one KPC-3 positive isolate was colistin resistant (EUCAST 2011 criteria). *Escherichia coli* ATCC 25922 was used as a quality control strain.

Multi Locus Sequence Typing showed that the ST258 clone was typical of the KPC-2 producing strains, while the ST512 clone was characteristic of the 5 *bla*KPC-3 producing strains.

The ST512 clone was spread in three acute care hospitals: “Niguarda Cà Granda” and IRCCS Istituto Clinico “Humanitas” (Milan) and “Alessandro Manzoni” (Lecco). The 5 ST512 strains were obtained from different specimens at neurological (n=3), medical (n=1) and onco-haematologic (n=1) units.

Using >95% similarity to define a clone, we detected a single cluster for all the KPC-3 produc-

ing *K. pneumoniae* isolates by rep-PCR-DiversiLab System (bioMérieux) (Figure 1). We highlight the *K. pneumoniae* ST512 clone intra- and inter-hospital spread in Northern Italy. The accurate detection of this emerging public health threat clone is crucial both for the selection of appropriate therapeutic regimens and for controlling the spread of KPC-type enzymes.

## REFERENCES

- CANTÓN R., AKÓVA M., CARMELI Y., GISKE C.G., GLUPCZYNSKI Y., GNIADKOWSKI M., LIVERMORE D.M., MIRIAGOU V., NAAS T., ROSSOLINI G.M., SAMUELSEN Ø., SEIFERT H., WOODFORD N., NORDMANN P.; EUROPEAN NETWORK ON CARBAPENEMASES. (2012). Rapid evolution and spread of carbapenemases among *Enterobacteriaceae* in Europe. *Clin. Microbiol. Infect.* **18**, 413-431.
- GIANI T., D’ANDREA M.M., PECILE P., BORGIANNI L., NICOLETTI P., TONELLI F., BARTOLONI A., ROSSOLINI G.M. (2009). Emergence in Italy of *Klebsiella pneumoniae* sequence type 258 producing KPC-3 Carbapenemase. *J. Clin. Microbiol.* **47**, 3793-3794.
- SAMUELSEN Ø., NASEER U., TOFTELAND S., SKUTLABERG D.H., ONKEN A., HJETLAND R., SUNDSFJORD A., GISKE C.G. (2009). Emergence of clonally related *Klebsiella pneumoniae* isolates of sequence type 258 producing plasmid-mediated KPC carbapenemase in Norway and Sweden. *J. Antimicrob. Chemother.* **63**, 654-658.