

# Successful containment and infection control of a Carbapenem-resistant *Klebsiella pneumoniae* outbreak in an Italian hospital

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## SUMMARY

We describe an outbreak of a carbapenemase-producing *Klebsiella pneumoniae* sequence type 258 (ST258) clone in an Italian hospital during two months in 2010. The rapid detection and management of the eleven patients colonized and infected with KPC-producing *K. pneumoniae* curbed the spread of this multidrug-resistant organism.

**KEY WORDS:** Infection control, KPC, *Klebsiella pneumoniae*.

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Carbapenem-resistant *Enterobacteriaceae* (CRE) are increasingly prevalent pathogens worldwide and represent a serious public health threat (Nordmann *et al.*, 2011). CRE infections (in particular bloodstream and low respiratory tract infections) have been associated with severe outcome (Zarkotou *et al.*, 2011; Lee *et al.*, 2012). High mortality rates of CRE infections are mainly due to the great limitation of antimicrobial therapy choice (Lee *et al.*, 2012). Numerous studies reported the widescale spread of different types of carbapenemases in Europe (Canton *et al.*, 2012). In particular, carbapenem resistance among *Enterobacteriaceae* in Italy are almost totally referable to the production of two carbapenemases types: class A, *K. pneumoniae* carbapenemase (KPC), and class B, metallo-beta-lactamase (MBL) (Ambretti *et*

*al.*, 2010; Gaibani *et al.*, 2011a; Gaibani *et al.*, 2011b; Gaibani *et al.*, 2013). Several strategies have been adopted to control CRE outbreaks (Owens *et al.*, 2006). Here, we described an outbreak of KPC-producing *K. pneumoniae* occurring in a large Italian hospital and the impact of timely laboratory diagnosis of KPC infections to the successful infection control measures adopted to control and manage the spread of this CRE.

On 8<sup>th</sup> November, a carbapenems-non susceptible *K. pneumoniae* was isolated from a patient admitted to the Intensive Care Unit (ICU) ward. In detail, the *K. pneumoniae* carbapenems-non susceptible isolate was analyzed for the antibiotic susceptibility using a Vitek2 semi-automated system (bioMérieux, France). Species identification was also performed by MALDI-TOF assay. Antibiogram revealed that the *K. pneumoniae* isolate was resistant to all beta-lactam antibiotics (including carbapenems), aminoglycosides, and fluoroquinolones, whereas it was susceptible only to gentamicin, tigecycline and colistin, in accordance with the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (The European Com-

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mittee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 3.0, 2013. <http://www.eucast.org>).

Subsequently, the modified Hodge Test (MHT) was performed as a confirmatory test (CLSI-MHT) for carbapenemase producers. The *K. pneumoniae* isolate gave positive results for the MHT assay, suggesting carbapenemase production. In order to discriminate among the specific class of carbapenemase types, the carbapenemase-producing *K. pneumoniae* was tested by a disc diffusion synergy test (DDST), as previously described (Ambretti *et al.*, 2013). The discriminatory carbapenemase assay showed a class A production. Polymerase chain reaction (PCR) assay for the *bla<sub>SHV</sub>*, *bla<sub>TEM</sub>*, *bla<sub>CTX</sub>* and *bla<sub>KPC</sub>* genes was performed to confirm the presence

of ESBLs and carbapenemases genes (Ambretti *et al.*, 2013). The amplicons were sequenced bi-directionally and the aligned sequences were compared by using the online BLAST database and CLUSTAL W software (version 2, available at: <http://www.ebi.ac.uk/clustalw2>). PCR and sequencing of the KPC-producing *K. pneumoniae* identified ESBL genes (*bla<sub>TEM-1</sub>*, *bla<sub>SHV-1</sub>*) and the carbapenemase *bla<sub>KPC-2</sub>* gene.

Based on the microbiologic results, rapid and efficient infection control measures were implemented by the Committee for the Control of Healthcare-related Infections of the Cà'Granda Hospital, in order to curb the spread of this MDR *K. pneumoniae*.

Since the detection of first KPC-producing *K. pneumoniae*, 11 carbapenems-non-susceptible *Enterobacteriaceae* have been isolated from 11

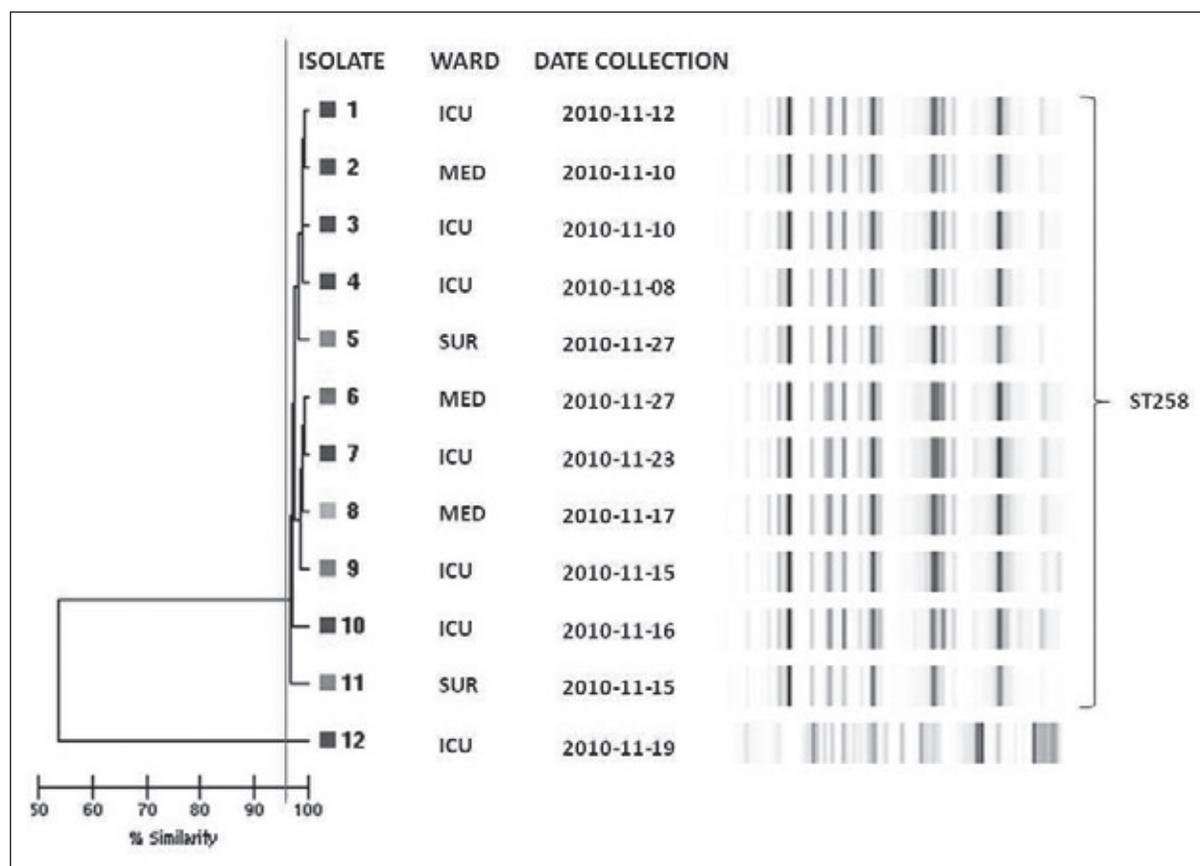


FIGURE 1 - RepPCR showing the genetic relationship of KPC-harboring *K. pneumoniae* strains isolated from different wards between 8th and 27th November 2010 at the Cà Granda Maggiore "Mangiagalli Regina Elena" Hospital, Milan. The dendrogram includes 11 KPC-2 producing *K. pneumoniae* isolates and a carbapenem-susceptible *K. pneumoniae* isolate, used as out-group. Isolates with  $\geq 95\%$  similarity were considered related. The MLST result is shown. Ward abbreviations: ICU, Intensive Care Unit; MED, Medical; SUR, Surgical.

patients hospitalized in the Cà Granda Ospedale Maggiore Hospital in Milan (Northern Italy) up to 19 days after the detection of the index case.

Four out of 11 patients were hospitalized in the ICU. All 11 *K. pneumoniae* gave positive results for the MHT assay, and the discriminatory carbapenemase assay showed a class A production, while no MBL were found. Antimicrobial susceptibility testing of the 11 isolates showed for all the isolates a multidrug-resistant (MDR) phenotype, whereas the susceptibility was limited only to gentamicin, and colistin (40% and 83.3% respectively) according to breakpoints from the EUCAST.

Since the index case detection, infection control operators have stressed the need to take selected epidemiological measures: isolation of positive patients, improvement of healthcare workers behaviors, including in particular reinforcement of hand hygiene. During the following two months no KPC-producers were detected during the active surveillance program implemented in different wards where infected and colonized patients were hospitalized.

Genotypes of the *enterobacteriaceae* isolates were determined by multilocus sequence typing (MLST) based on seven housekeeping genes (*rpoB*, *gapA*, *mdh*, *pgi*, *phoE*, *InfB*, *tonB*) as previously described (Diacourt *et al.*, 2005). To investigate the genomic relatedness of the *K. pneumoniae* isolates, a semi-automated repetitive element PCR (rep-PCR) technique (DiversiLab™; bioMérieux) was carried out. The rep-PCR technique has been indicated for proper outbreak management due to the same discriminatory ability as the Pulsed-field gel electrophoresis (PFGE) technique with the advantage of a faster turnaround result (Cuzon *et al.*, 2012).

RepPCR genotyping of the 11 KPC-producing *K. pneumoniae* revealed identical profiles, indicating that all isolates were clonally related (Figure 1). MLST analysis, carried out with three randomly selected isolates from different patients, revealed that they belonged to sequence type ST258.

Since 2010, the rapid increase in KPC-producing *enterobacteriaceae* has been observed worldwide and especially in Europe (Canton *et al.*, 2012). Consequently, the rapid detec-

tion of carbapenemase-producing bacteria is a fundamental key in the management and control of their diffusion (Nordmann *et al.*, 2012; Owens *et al.*, 2006). Our report showed a rapid and accurate identification of an outbreak of KPC-harboring *K.pneumoniae* in a large Italian hospital. Based on these findings, the implementation of microbiological methods for the detection of carbapenemase producers should be considered a primary tool for any surveillance protocol designed to promptly identify the spread of carbapenem-resistant *Enterobacteriaceae* allowing all infection control measures, including healthcare workers re-training and improvement of hand hygiene, to be reinforced.

## REFERENCES

- AMBRETTI S., GAIBANI P., BERLINGERI A., CORDOVANA M., TAMBURINI M.V., BUA G., LANDINI M.P., SAMBRI V. (2013). Evaluation of phenotypic and genotypic approaches for the detection of class A and class B carbapenemases in enterobacteriaceae. *Microb. Drug. Resist.* In Press.
- AMBRETTI S., GAIBANI P., CAROLI F., MIRAGLIOTTA L., SAMBRI V. (2010). A carbapenem-resistant *Klebsiella pneumoniae* isolate harboring KPC-1 from Italy. *New Microbiol.* **33**, 281-282.
- CANTÓN R, AKÓVA M., CARMELI Y., GISKE C.G., GLUPCZYNSKI Y., GNIADKOWSKI M., LIVERMORE D.M., MIRAGOU V., NAAS T., ROSSOLINI G.M., SAMUELSEN Ø., SEIFERT H., WOODFORD N., NORDMANN P. (2012). Rapid evolution and spread of carbapenemases among Enterobacteriaceae in Europe. *Clin. Microbiol. Infect.* **18**, 413-431.
- CUZON G., NAAS T., DEMACHY M.C., NORDMANN P. (2012). Nosocomial outbreak of *Klebsiella pneumoniae* harbouring bla(KPC-3) in France subsequent to a patient transfer from Italy. *Int. J. Antimicrob. Agents.* **39**, 448-449.
- DIANCOURT L., PASSET V., VERHOEF J., GRIMONT P.A., BRISSE S. (2005). Multilocus sequence typing of *Klebsiella pneumoniae* nosocomial isolates. *J. Clin. Microbiol.* **43**, 4178-4182.
- GAIBANI P., AMBRETTI S., BERLINGERI A., CORDOVANA M., FARRUGIA P., PANICO M., LANDINI M.P., SAMBRI V. (2011a). Outbreak of NDM-1-producing Enterobacteriaceae in northern Italy, July to August 2011. *Euro Surveill.* **16**, pii20027.
- GAIBANI P., AMBRETTI S., BERLINGERI A., GELSOMINO F., BIELLI A., LANDINI M.P., SAMBRI V. (2011b). Rapid increase of carbapenemase-producing *Klebsiella pneumoniae* strains in a large Italian hospital:

- surveillance period 1 March - 30 September 2010. *Euro Surveill.* **16**, pii:19800.
- GAIBANI P, AMBRETTI S, FARRUGLIA P, BUA G, BERLINGERI A, TAMBURINI MV, CORDOVANA M, GUERRA L, MAZZETTI M, RONCARATI G, TENACE C, MORO ML, GAGLIOTTI C, LANDINI MP, SAMBRI V. (2013). Outbreak of *Citrobacter freundii* carrying VIM-1 in an Italian Hospital, identified during the carbapenemases screening actions, June 2012. *Int. J. Infect. Dis.* pii: S1201-9712 (13) 00097-0.
- LEE G.C., BURGESS D.S. (2012). Treatment of *Klebsiella Pneumoniae* Carbapenemase (KPC) infections: a review of published series and cases. *Ann. Clin. Microbiol. Antimicrob.* **11**, 32.
- NORDMANN P, NAAS T, POIREL L. (2011). Global spread of Carbapenemase-producing Enterobacteriaceae. *Emerg. Infect. Dis.* **17**, 1791-1798.
- OWENS R.C. JR., RICE L. (2006). Hospital-based strategies for combating resistance. *Clin. Infect. Dis.* **42**, S173-181.
- ZARKOTOU O., POURNARAS S., TSELIOTI P., DRAGOMANOS V., PITIRIGA V., RANELLOU K., PREKATES A., THEMELIDIGALAKI K., TSAKRIS A. (2011). Predictors of mortality in patients with bloodstream infections caused by KPC-producing *Klebsiella pneumoniae* and impact of appropriate antimicrobial treatment. *Clin. Microbiol. Infect.* **17**, 1798-1803.