

Colistin resistance superimposed to endemic carbapenem-resistant *Klebsiella pneumoniae*: a rapidly evolving problem in Italy, November 2013 to April 2014

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Consecutive non-replicate clinical isolates (n=191) of carbapenem non-susceptible Enterobacteriaceae were collected from 21 hospital laboratories across Italy from November 2013 to April 2014 as part of the European Survey on Carbapenemase-producing Enterobacteriaceae (EuSCAPE) project. *Klebsiella pneumoniae* carbapenemase-producing *K. pneumoniae* (KPC-KP) represented 178 (93%) isolates with 76 (43%) respectively resistant to colistin, a key drug for treating carbapenemase-producing Enterobacteriaceae. KPC-KP colistin-resistant isolates were detected in all participating laboratories. This underscores a concerning evolution of colistin resistance in a setting of high KPC-KP endemicity.

We report the widespread and rapid dissemination of resistance against colistin, a key drug for treatment of carbapenemase-producing Enterobacteriaceae, among *Klebsiella pneumoniae* carbapenemase (KPC)-producing *K. pneumoniae* (KPC-KP) in Italy. As part of the European Survey on Carbapenemase-producing Enterobacteriaceae (EuSCAPE) project, consecutive non-replicate clinical isolates of carbapenem non-susceptible (resistant or intermediate) Enterobacteriaceae (n=191) were collected from 21 Italian hospital laboratories between November 2013 and April 2014. Most isolates 178 (93%) were KPC-KP, with 76 (43%) respectively resistant to colistin. This report details the findings and discusses potential implications for infection control.

Background

Carbapenem-resistant Enterobacteriaceae (CRE) emerged in recent years as one of the most challenging group of antibiotic-resistant pathogens. Related

mortality rates are high due to limited treatment options, and some strains have the potential for rapid dissemination in healthcare settings [1,2]. In Europe, CRE have been reported from virtually all countries, but in some countries, namely Greece and Italy, they have spread rapidly and are presently endemic in many hospitals [3,4]. Resistance to carbapenems in Enterobacteriaceae is largely due to production of enzymes (carbapenemases) inactivating these antibiotics, hence the definition of carbapenemase-producing Enterobacteriaceae (CPE).

In Italy, the dramatic increase of carbapenem-resistant *Klebsiella pneumoniae* has been documented by the European Antimicrobial Resistance Surveillance Network (EARS-Net) which showed that the percentage of invasive isolates of carbapenem-resistant *K. pneumoniae*, that was until 2009 lower than one to 2%, increased to 15% in 2010 to reach 35% in 2013 ([5] and unpublished data). Data provided by Micronet (<http://www.simi.iss.it/micronet.htm>), a sentinel epidemiological surveillance network based on computerised daily collection of microbiological data from the laboratory information systems of 27 laboratories nationwide, confirmed the increase in the percentage of carbapenem-resistant *K. pneumoniae* in samples from different anatomical sites, including lower respiratory secretions and urine [6]. In addition, analysis of resistance determinants and clonality, revealed that the Italian CRE epidemic was mostly sustained by KPC-KP of clonal complex 258, with only a minority of different clones and resistance mechanisms [7].

Polymyxins (colistin and polymyxin B), together with tigecycline and gentamicin, are among the few agents

TABLE

Carbapenemase determinants detected in the confirmed carbapenem non-susceptible isolates collected as part of the EuSCAPE survey, Italy, November 2013–April 2014 (n=191)

Species	Number of isolates per type of carbapenemase				
	<i>bla</i> _{KPC}	<i>bla</i> _{VIM}	<i>bla</i> _{NDM}	<i>bla</i> _{OXA-48}	None detected
<i>Klebsiella pneumoniae</i>	178 ^a	3	1	1	4
<i>Escherichia coli</i> ^b	3	1	–	–	–

EuSCAPE: European Survey on Carbapenemase-producing Enterobacteriaceae.

K. pneumoniae carbapenemase-producing *K. pneumoniae* (KPC-KP) were reported from all peripheral laboratories.

^a Detected from all the 21 peripheral laboratories.

^b The four carbapenemase-producing *E. coli* isolates were from different peripheral laboratories.

that retain activity against KPC-KP, and are key components of the combination antimicrobial regimens that are recommended for treatment of these pathogens [8,9]. Therefore, the emergence of resistance to these last line drugs among KPC-KP is important to monitor.

Implementation of European Survey on Carbapenemase-producing Enterobacteriaceae in Italy

EuSCAPE is funded by the European Centre for Disease Prevention and Control (ECDC) and coordinated by the Department of Medical Microbiology of the University Medical Center Groningen in the Netherlands. This initiative aims to foster active surveillance of CPE through improving the diagnostic capacity of microbiological laboratories in Europe [10]. A crucial part of EuSCAPE consisted of a structured survey that between November 2013 and April 2014 involved hospital laboratories from 35 countries across Europe. In each participating country the National Expert Laboratory (NEL) collected and characterised clinical isolates of suspected carbapenem non-susceptible *K. pneumoniae* or *Escherichia coli* obtained from a sentinel network of peripheral laboratories (PLs). Each PL was asked to collect the first 10 consecutive non-replicate isolates of suspected carbapenem non-susceptible *K. pneumoniae* or *E. coli* obtained from clinical samples (blood, lower respiratory tract secretions, urine, puncture fluids and wound secretions) and to provide also relevant demographic and clinical data (age, sex, location of patient in hospital, previous hospital admission in the last six months, previous stay or travel abroad within the last six months).

In Italy, a total of 21 PLs that served 45 hospitals or outpatients clinics distributed across the country participated in the survey. PLs identified suspected carbapenem non-susceptible *K. pneumoniae* or *E. coli* by automated systems Vitek 2 (bioMérieux, Marcy l'Etoile, France) or Phoenix (Becton Dickinson Diagnostic Systems, Sparks, MD, USA). Subsequently these isolates were sent to the NEL in Rome, who in collaboration with the NEL in Siena, performed confirmation and further characterisation. NELs confirmed species identification by matrix-assisted laser desorption/ionization

time-of-flight (MALDI-TOF) mass spectrometry (Vitek MS, bioMérieux), and carried out susceptibility testing against carbapenems and other antimicrobial agents by reference broth microdilution [11] using commercial microtitre plates (Alere Technologies, GmbH, Jena, Germany) and manually prepared plates for colistin testing. Results were interpreted according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) clinical breakpoints [12]. The presence of carbapenemase genes of the *bla*_{KPC}, *bla*_{NDM}, *bla*_{VIM}, and *bla*_{OXA-48} types was investigated by polymerase chain reaction (PCR) using the protocol recommended by EuSCAPE (available upon request from the EuSCAPE Coordinator, Prof. Hajo Grundmann).

Results of the survey

A total of 197 suspected carbapenem non-susceptible *K. pneumoniae* or *E. coli* isolates were collected by the PLs in the study period. Of these, 187 *K. pneumoniae* and four *E. coli* were confirmed as non-susceptible to at least one carbapenem antibiotic (imipenem, meropenem or ertapenem). The *bla*_{KPC} determinant was found to be the most prevalent among carbapenem non-susceptible isolates, being detected in 178 *K. pneumoniae* and in three *E. coli*, while other carbapenemase genes were infrequently found (Table).

KPC-KP were obtained from urine (67 isolates), blood (61 isolates), lower respiratory tract (21 isolates), wound secretions (10 isolates), and other specimens (19 isolates). Patients with KPC-KP had a median age of 72 years (range: 16–94 years); 106 (60%) were males and 72 (40%) were females. Of these patients, 41 (23%) had KPC-KP isolates detected while in intensive care unit (ICU), 127 (71%) were found while in a medical or surgical ward, and 10 (6%) were outpatients or patients seen at the emergency department. Another hospital admission in the previous six months was reported for 96 (64%) of patients for whom the information was available (n=150). Travelling abroad during the last six months was reported for only 3 (3%) of the patients for whom the information was available (n=111). Thus, 97% (108/111) of KPC-KP infections are endemic cases.

FIGURE

Distribution of peripheral laboratories reporting KPC-producing *K. pneumoniae* isolates in the EuSCAPE Italian survey, Italy, November 2013–April 2014 (n=191 isolates)



EuSCAPE: European Survey on Carbapenemase-producing Enterobacteriaceae; KPC: *Klebsiella pneumoniae* carbapenemase; KPC-KP: KPC-producing *K. pneumoniae*.

The peripheral laboratories are numbered on the map according to alphabetical order.

Proportions of colistin-resistant isolates among KPC-KP per peripheral laboratory: 1, Alessandria: 1/10; 2, Ancona: 8/10; 3, Ferrara: 1/4; 4, Florence: 5/10; 5, Foggia: 4/10; 6, Lecco: 2/9; 7, Milan: 1/10; 8, Modena: 3/7; 9, Naples: 3/8; 10, Perugia: 5/10; 11, Reggio Calabria: 4/10; 12, Rome: 4/9; 13, Rome: 2/4; 14, Rome: 6/7; 15, San Remo: 4/8; 16, Siena: 6/8; 17, Treviso: 1/7; 18, Turin: 5/9; 19, Udine: 2/8; 20, Venice: 8/10; 21, Vercelli: 1/10.

Antimicrobial susceptibility data for the 178 KPC-KP isolates revealed that 76 (43%) were resistant to colistin, 11 (6%) resistant or intermediate to tigecycline, 29 (16%) resistant or intermediate to gentamicin, and 146 (82%) resistant or intermediate to trimethoprim-sulfamethoxazole (SXT). Two isolates (1%) were resistant or intermediate to all four antibiotics. Colistin-resistant KPC-KP isolates were detected from all PLs, although at variable percentages (Figure).

Discussion and conclusions

Although most recent data from April 2014 to date are not available at this time, the results of this survey confirmed the widespread endemicity of KPC-KP in Italian healthcare facilities, and their predominant role among CPE. Infections with KPC-KP affect mostly older

patients hospitalised in medical or surgical wards with a known history of previous hospital admission in the country. The results of this present study also reveal a concerning percentage of resistance to colistin, which is a matter of major concern given the dearth of treatment options against CPE.

In Italy, the emergence of colistin-resistant KPC-KP has been reported since 2010 [13] and, in the first Italian nationwide cross-sectional survey on CRE, carried out in mid-2011, the overall percentage of colistin resistance among KPC-KP was found to be 22.4%, with colistin-resistant isolates reported from 13 of 25 participating hospital laboratories [7]. In the EuSCAPE study, the colistin resistance percentage found among KPC-KP was almost double, and colistin-resistant KPC-KP isolates were detected from all 21 PLs in the study. We did not have information to derive the total number of affected hospitals among the 45 served by the 21 PLs, however the PLs were distributed all across the country. A similar situation of nationwide dissemination of colistin-resistant KPC-KP has not yet been reported in other settings of high KPC-KP endemicity [14].

According to data available from the European Surveillance of Antimicrobial Consumption Network (ESAC-NET) database [15], consumption of polymixins in the hospital sector in Italy increased from 0.0017 to 0.0194 Defined Daily Dose (DDD) per 1,000 inhabitants per day in the period from 2007 to 2012. This 10-fold increase reflects the increasing dissemination of multidrug-resistant Gram-negative infections for which colistin remains one of the few therapeutic options and most likely contributed to selection of colistin-resistant strains among KPC-KP.

To control the spread of KPC-KP in Italy, in February 2013 the Ministry of Health issued a circular letter [16] asking the Italian regions to report all cases of bloodstream infections due to CPE of the species *K. pneumoniae* or *E. coli* and recommending control measures to limit the spread in healthcare settings. These control measures consist of: (i) active screening of selected patient groups including patients who have been in contact with CPE-colonised or infected patients, and patients coming from countries with high CPE endemicity and, if feasible, patients admitted to ICU or other high-risk wards and patients with a history of previous hospitalisation; (ii) isolation or cohorting of infected/colonised patients, separate cohort nursing care, and implementation of contact precautions, according to the recommendations issued at national and international level [17-20].

These measures require huge efforts and resources in an endemic situation like the one highlighted in this study, since patients with KPC-KP infection or colonisation are not confined to ICUs, but can be found in normal hospital wards. It seems therefore urgent to develop and implement a national plan for the prevention and control of CPE infections in Italy that includes

an extensive surveillance system and more comprehensive guidelines on infection control measures. Sufficient resources should be allocated to contain the further dissemination of CPE in healthcare institutions.

CCHFV was isolated in vitro from the PCR-positive serum sample within the Containment Level 4 laboratory at PHE Porton, using the human SW13 cell line.

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Conflict of interest

MM, MR, AGF, HG and AP have nothing to declare; GMR has received research grants from Pfizer, Astra-Zeneca, Cubist, Angelini, Becton-Dickinson, bioMérieux, Biotest, VenatoRx, has served as consultant for Pfizer, Astra-Zeneca, Cubist, Angelini, Menarini, Achaogen, Rempex, Durata, Medivir, Biotest, and has served in the Speaker's Bureau for Pfizer, Astra-Zeneca, Novartis, Angelini, Curetis, Biotest and Basilea. TG has served in the Speaker's Bureau for bioMérieux.

Author contributions

MM, TG, AP and GMR contributed to the design of the study, to draft and finalise the manuscript; TG, SP, FA performed the phenotypic characterisation of the isolates; MM, MR, AGF performed the detection of the carbapenemase genes by PCR; MM, MR, AGF, TG, SP, FA entered and analysed data; HG planned and coordinated the EuSCAPE study and revised the final manuscript; PLs provided isolates and clinical and demographic data of patients.

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References

1. Tzouveleki LS, Markogiannakis A, Psychogiou M, Tassios PT, Daikos GL. Carbapenemases in *Klebsiella pneumoniae* and other Enterobacteriaceae: an evolving crisis of global dimensions. *Clin Microbiol Rev.* 2012;25(4):682-707. <http://dx.doi.org/10.1128/CMR.05035-11>
2. Nordmann P, Naas T, Poirel L. Global spread of carbapenemase-producing Enterobacteriaceae. *Emerg Infect Dis.* 2011;17(10):1791-8. <http://dx.doi.org/10.3201/eid1710.110655>
3. Glasner C, Albiger B, Buist G, Tambić Andrašević A, Canton R, Carmeli Y, et al. Carbapenemase-producing Enterobacteriaceae in Europe: a survey among national experts from 39 countries, February 2013. *Euro Surveill.* 2013;18(28):pii=20525.
4. Cantón R, Akóva M, Carmeli Y, Giske CG, Glupczynski Y, Gniadkowski M, et al. Rapid evolution and spread of carbapenemases among Enterobacteriaceae in Europe. *Clin Microbiol Infect.* 2012;18(5):413-31. <http://dx.doi.org/10.1111/j.1469-0691.2012.03821.x>
5. European Antimicrobial Resistance Surveillance Network (EARS-Net). Stockholm: ECDC. [Accessed 21 Oct 2014]. Available from: <http://www.ecdc.europa.eu/en/activities/surveillance/EARS-Net/Pages/index.aspx>
6. Sisto A, D' Ancona F, Meledandri M, Pantosti A, Rossolini GM, Raglio A, et al. Carbapenem non-susceptible *Klebsiella pneumoniae* from Miconet network hospitals, Italy, 2009 to 2012. *Euro Surveill.* 2012;17(33):pii=20247.
7. Giani T, Pini B, Arena F, Conte V, Bracco S, Migliavacca R, et al. Epidemic diffusion of KPC carbapenemase-producing *Klebsiella pneumoniae* in Italy: results of the first countrywide survey, 15 May to 30 June 2011. *Euro Surveill.* 2013;18(22):pii=20489.
8. Petrosillo N, Giannella M, Lewis R, Viale P. Treatment of carbapenem-resistant *Klebsiella pneumoniae*: the state of the art. *Expert Rev Anti Infect Ther.* 2013;11(2):159-77. <http://dx.doi.org/10.1586/eri.12.162>
9. Tzouveleki LS, Markogiannakis A, Piperaki E, Souli M, Daikos GL. Treating infections caused by carbapenemase-producing Enterobacteriaceae. *Clin Microbiol Infect.* 2014;20(9):862-72. <http://dx.doi.org/10.1111/1469-0691.12697>
10. European Centre for Disease Prevention and Control (ECDC). Carbapenemase-producing bacteria in Europe: interim results from the European Survey on carbapenemase-producing Enterobacteriaceae (EuSCAPE) project. Stockholm: ECDC; 2013.
11. Clinical and Laboratory Standards Institute (CLSI). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved 255 Standard. Eighth Edition. Wayne, PA: CLSI; document M7-A9.
12. European Committee on Antimicrobial Susceptibility Testing (EUCAST). Clinical breakpoints. Växjö: EUCAST. [Accessed 21 Oct 2014]. Available from: http://www.eucast.org/clinical_breakpoints/
13. Mezzatesta ML, Gona F, Caio C, Petrolito V, Sciortino D, Sciacca A, et al. Outbreak of KPC-3-producing, and colistin-resistant, *Klebsiella pneumoniae* infections in two Sicilian hospitals. *Clin Microbiol Infect.* 2011;17(9):1444-7.
14. Ah YM, Kim AJ, Lee JY. Colistin resistance in *Klebsiella pneumoniae*. *Int J Antimicrob Agents.* 2014;44(1):8-15. <http://dx.doi.org/10.1016/j.ijantimicag.2014.02.016>
15. European Centre for Disease Prevention and Control (ECDC). Consumption of antimicrobials of Antibacterials For Systemic Use (ATC group J01) in the community (primary care sector) in Europe, reporting year 2012. Stockholm: ECDC. [Accessed 21 Oct 2014]. Available from: http://www.ecdc.europa.eu/en/healthtopics/antimicrobial_resistance/esac-net-database/Pages/Antimicrobial-consumption-rates-by-country.aspx
16. Ministero della Salute. Circolare 'Sorveglianza e controllo delle infezioni da batteri produttori di carbapenemasi (CPE)'. [Circular letter 'Surveillance and control of infections due to carbapenemase-producing bacteria (CPE)']. Rome: Ministero della Salute; Feb 2014. Italian. [Accessed 21 Oct 2014]. Available from: <http://www.trovanorme.salute.gov.it/norme/renderNormsanPdf?anno=0&codLeg=45499&parte=1%20&serie=>
17. Grundmann H, Livermore DM, Giske CG, Canton R, Rossolini GM, Campos J, et al. Carbapenem-non-susceptible

- Enterobacteriaceae in Europe: conclusions from a meeting of national experts. *Euro Surveill.* 2010;15(46):pii=19711.
18. Schwaber MJ, Lev B, Israeli A, Solter E, Smollan G, Rubinovitch B, et al. Containment of a country-wide outbreak of carbapenem-resistant *Klebsiella pneumoniae* in Israeli hospitals via a nationally implemented intervention. *Clin Infect Dis.* 2011;52(7):848-55. <http://dx.doi.org/10.1093/cid/cir025>
 19. Akova M, Daikos GL, Tzouveleki L, Carmeli Y. Interventional strategies and current clinical experience with carbapenemase-producing Gram-negative bacteria. *Clin Microbiol Infect.* 2012;18(5):439-48. <http://dx.doi.org/10.1111/j.1469-0691.2012.03823.x>
 20. Tacconelli E, Cataldo MA, Dancer SJ, De Angelis G, Falcone M, Frank U, et al. ESCMID guidelines for the management of the infection control measures to reduce transmission of multidrug-resistant Gram-negative bacteria in hospitalized patients. *Clin Microbiol Infect.* 2014;20(Suppl 1):1-55. <http://dx.doi.org/10.1111/1469-0691.12427>