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The changing epidemiology of carbapenemase-producing *Klebsiella pneumoniae* in Italy: toward polyclonal evolution with emergence of high-risk lineages

Vincenzo Di Pilato [1]†‡, Giulia Errico[2,3]†, Monica Monaco[2], Tommaso Giani[1,4], Maria Del Grosso[2], Alberto Antonelli [1], Sophia David[5], Erika Lindh[2,3], Romina Camilli[2], David M. Aanensen[5,6], Gian Maria Rossolini[1,4] and Annalisa Pantosti[2]* on behalf of the AR-ISS Laboratory Study Group on carbapenemase-producing *Klebsiella pneumoniae*§

[1]*Department of Experimental and Clinical Medicine, University of Florence, Florence, Italy;* [2]*Department of Infectious Diseases, Istituto Superiore di Sanità, Rome, Italy;* [3]*European Program for Public Health Microbiology Training (EUPHEM), European Centre for Disease Prevention and Control, (ECDC), Stockholm, Sweden;* [4]*Clinical Microbiology and Virology Unit, Careggi University Hospital, Florence, Italy;* [5]*Centre for Genomic Pathogen Surveillance, Wellcome Sanger Institute, Cambridge, UK;* [6]*Big Data Institute, Li Ka Shing Centre for Health Information and Discovery, Nuffield Department of Medicine, University of Oxford, Oxford, UK*

*Corresponding author. E-mail: annalisa.pantosti@iss.it
†Vincenzo Di Pilato and Giulia Errico contributed equally to this study and both should be considered first author.
‡Present address: Department of Surgical Sciences and Integrated Diagnostics, University of Genoa, Genoa, Italy.
§Other members of the AR-ISS Study Group on carbapenemase-producing *Klebsiella pneumoniae* are listed in the Acknowledgements section.

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Background: Previous studies showed that the epidemic of carbapenem-resistant *Klebsiella pneumoniae* (CR-KP) observed in Italy since 2010 was sustained mostly by strains of clonal group (CG) 258 producing KPC-type carbapenemases. In the framework of the National Antibiotic-Resistance Surveillance (AR-ISS), a countrywide survey was conducted in 2016 to explore the evolution of the phenotypic and genotypic characteristics of CR-KP isolates.

Methods: From March to July 2016, hospital laboratories participating in AR-ISS were requested to provide consecutive, non-duplicated CR-KP (meropenem and/or imipenem MIC >1 mg/L) from invasive infections. Antibiotic susceptibility was determined according to EUCAST recommendations. A WGS approach was adopted to characterize the isolates by investigating phylogeny, resistome and virulome.

Results: Twenty-four laboratories provided 157 CR-KP isolates, of which 156 were confirmed as *K. pneumoniae sensu stricto* by WGS and found to carry at least one carbapenemase-encoding gene, corresponding in most cases (96.1%) to bla_{KPC}. MLST- and SNP-based phylogeny revealed that 87.8% of the isolates clustered in four major lineages: CG258 (47.4%), with ST512 as the most common clone, CG307 (19.9%), ST101 (15.4%) and ST395 (5.1%). A close association was identified between lineages and antibiotic resistance phenotypes and genotypes, virulence traits and capsular types. Colistin resistance, mainly associated with *mgrB* mutations, was common in all major lineages except ST395.

Conclusions: This WGS-based survey showed that, although CG258 remained the most common CR-KP lineage in Italy, a polyclonal population has emerged with the spread of the new high-risk lineages CG307, ST101 and ST395, while KPC remained the most common carbapenemase.

Introduction

According to estimates elaborated by the ECDC, over 200 000 infections and nearly 11 000 deaths due to antibiotic-resistant bacterial pathogens occurred in Italy in 2015, with a large proportion of the total burden caused by carbapenem- or colistin-resistant Gram-negative species (*Escherichia coli*, *Klebsiella pneumoniae*, *Acinetobacter* spp. and *Pseudomonas aeruginosa*).[1]

During the past decade, carbapenem-resistant *K. pneumoniae* (CR-KP) emerged and spread worldwide as a major antibiotic-resistant threat, causing infections associated with high mortality rates,[2] and becoming endemic in many countries across

Europe.[3–5] In Italy, the proportion of CR-KP among invasive isolates rose dramatically from 1% in 2009 to 15% in 2010 and to 34% in 2016, with a slight decrease (27%) in 2018.[6]

In the early phases, the CR-KP epidemic observed in Italy was found to be mostly associated with the expansion of high-risk clones of ST512 and ST258, included in the clonal group (CG) 258, producing KPC-type carbapenemases.[3,7–10] Later epidemiological studies have shown the emergence of other KPC-producing high-risk clones, such as ST307, outcompeting CG258 in some geographical areas.[11–13]

In this study, we report the results of the characterization of a nationwide collection of CR-KP invasive isolates obtained in the framework of the National Antibiotic-Resistance Surveillance (AR-ISS).[14] The genetic diversity of CR-KP isolates was explored by WGS, which is currently considered the gold standard for molecular epidemiology investigations.[15]

Methods

Study design and isolates

Thirty hospital laboratories participating in the AR-ISS, located in different areas of Italy (Figure 1), were enrolled to collect all consecutive, non-duplicated clinical isolates of *K. pneumoniae* from blood or CSF exhibiting a meropenem and/or imipenem MIC >1 mg/L, during the period March–July 2016. Isolates showing the requested criteria were defined as CR-KP for the purpose of this study.

Bacterial identification and *in vitro* susceptibility testing were performed by the participating hospital laboratories using routine methods.[3]

At reference laboratories (Istituto Superiore di Sanità and University of Florence), antimicrobial susceptibility testing was carried out by reference broth microdilution using lyophilized custom plates (Merlin Diagnostika, Germany), and by reference agar dilution for fosfomycin.[16] Results were interpreted according to the 2020 EUCAST clinical breakpoints (v. 10.0).[16] For tigecycline, results were interpreted using the *E. coli* breakpoints.[16]

WGS and bioinformatics analyses

Total genomic DNA was extracted from cultures using Qiagen QIAsymphony (QIAGEN, Hilden, Germany) and sent to the Wellcome Sanger Institute in Cambridge (UK) for WGS.

Shotgun libraries were prepared using the NEB Ultra II custom kit and were subjected to WGS with the HiSeq X10 platform (Illumina, Inc., San Diego, CA, USA), using a 2×150 bp paired-end approach. Sequence reads were processed as previously described.[17]

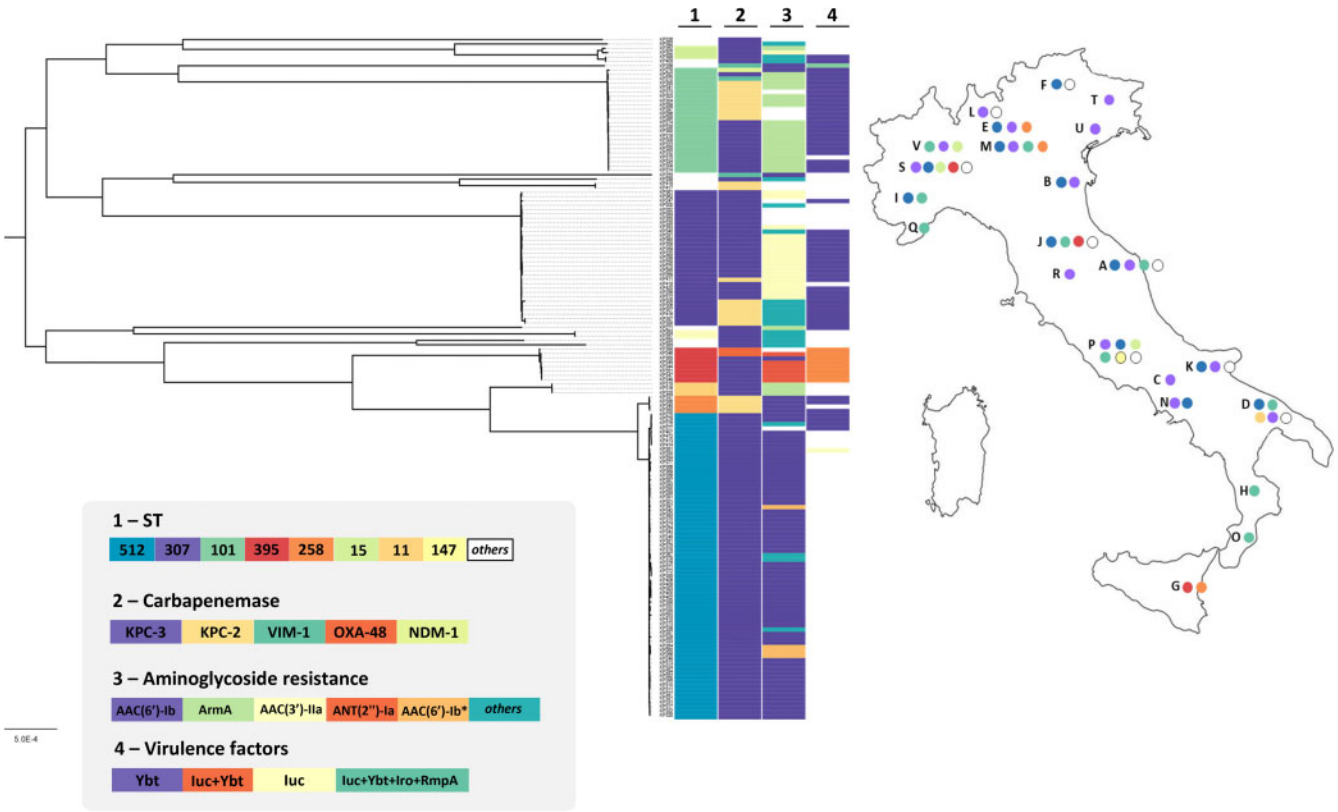

Figure 1. Genetic diversity of carbapenemase-producing *K. pneumoniae* isolates and distribution among the Italian laboratories. Left panel: core genome SNP-based phylogenetic tree of 156 *K. pneumoniae* isolates and main features, including ST, type of carbapenemase, aminoglycoside resistance determinants and virulence factors. For each group of features, sorted from the most to the least represented, a colour-based key is given. Right panel: Italian laboratories providing CR-KP isolates and distribution of the most common lineages, indicated by dots following the colours of the ST feature key. AAC(6′)-Ib*, corresponds to the L119S variant of AAC(6′)-Ib. Laboratory codes: A, Ancona; B, Area Vasta Romagna; C, Aversa; D, Bari; E, Bergamo; F, Bolzano; G, Catania; H, Cosenza; I, Cuneo; J, Florence; K, Foggia; L, Lecco; M, Milan (including M1, Legnano; M2, Milan IRCCS; M3, Milan Istituto Tumori); N, Naples; O, Reggio Calabria; P, Rome; Q, Sanremo; R, Siena; S, Turin; T, Udine; U, Venice; and V, Vercelli. This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

In silico analyses were performed using dedicated tools available at the Center for Genomic Epidemiology (https://cge.cbs.dtu.dk/). Sequence comparisons were performed using BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The global phylogenetic relatedness was investigated by generation of core genome SNP-based phylogenetic trees, using Roaryand IQ-TREE v1.6.12 applying a generalized time reversible (GTR) model.[18–20] The same analysis was performed on isolates belonging to the four major lineages CG258, CG307, ST101 and ST395. For the purpose of this study, only isolates belonging to ST258 and to single- or double-locus variants of ST258 carrying the *tonB79* MLST allele were included in CG258 (e.g. ST11 was excluded), in order to analyse only the most phylogenetically related members.[21] Strains belonging to ST307 and to the single-locus variant ST2975 were included in CG307. Capsular types and virulence loci were analysed using Kleborate (https://github.com/katholt/Kleborate).

Data availability

All raw sequences are available at the European Nucleotide Archive under the study accession number PRJEB22890. Individual accession numbers for *de novo* assemblies and genetic features of sequenced strains are detailed in Table S1 (available as Supplementary data at *JAC* Online).

Results and discussion

CR-KP isolates and antibiotic resistance profiles

In the period from March to July 2016, 24 out of 30 enrolled hospital laboratories isolated 157 CR-KP and sent them to the reference laboratories for phenotypic characterization and analysis. Six out of the 30 enrolled hospital laboratories did not isolate CR-KP. Each of the 24 laboratories collected a number of isolates ranging from 1 to 28. A similar number of CR-KP isolates was obtained from laboratories located in North, Central and South Italy (Table 1), ensuring a homogeneous geographical representation. By WGS, 156 CR-KP isolates were identified as *K. pneumoniae sensu stricto* and one as *Klebsiella variicola* that was not included in subsequent analysis.

Antibiotic susceptibility tests showed that resistance rates to carbapenems ranged from 91.7% to 100%. Most isolates were resistant to ciprofloxacin (97.5%), chloramphenicol (84.0%) and trimethoprim/sulfamethoxazole (71.8%), while lower resistance rates were observed to aminoglycosides (gentamicin, 40.4%; amikacin, 55.1%), tigecycline (49.4%) and colistin (40.4%) (Table 2). The rate of colistin resistance was similar to that previously reported by Monaco *et al.*[4] for CR-KP isolates collected in 2013–14. Since the use of colistin has recently been reduced due to the availability of novel antibiotic–inhibitor combinations to treat infections due to KPC-producing *K. pneumoniae* (KPC-KP),[22] further national surveys will be required to investigate the evolution of colistin resistance. Fosfomycin and ceftazidime/avibactam were the most active agents, with resistance rates of 17.3% and 2.6%, respectively (Table 2).

All isolates contained at least one carbapenemase-encoding gene, corresponding in most cases (96.1%) to bla_{KPC}, with a higher prevalence of bla_{KPC-3} (85.3%,) versus bla_{KPC-2} (14.7%) (Table 1 and Table S1). In one isolate, bla_{KPC-3} was detected together with bla_{OXA-48}. Among the remaining isolates, three (1.9%) were positive for bla_{VIM-1}, two (1.3%) for bla_{OXA-48} and one for both bla_{NDM-1} and bla_{OXA-48} (Figure 1 and Table 1). KPC-KP isolates were detected in all the hospital laboratories, confirming a countrywide endemicity in line with data previously reported by EuSCAPE[5,23] and by several Italian studies.[3,7] These results confirm that, 6 years after the start of the epidemic diffusion of CR-KP in Italy, the main mechanism of carbapenem resistance remained the production of KPC-type carbapenemases, while the presence of other carbapenemases was limited to sporadic cases.

Population structure and resistance patterns of the major CR-KP lineages

The core genome SNP-based phylogeny and the MLST analyses conducted on the 156 CR-KP isolates revealed the presence of 23 STs, of which three were newly assigned (ST3498, ST3499 and ST3500) (Table 1 and Table S1).

The majority of isolates (87.8%) clustered in four major lineages: CG258 ($n = 74$), mainly represented by ST512 ($n = 67$); CG307 ($n = 31$), mostly represented by ST307 ($n = 30$); ST101 ($n = 24$); and ST395 ($n = 8$). The remaining isolates belonged to ST11 ($n = 3$), ST15 ($n = 3$), ST147 ($n = 2$) and to 11 other STs (one isolate each) (Figure 1 and Table 1).

The four major lineages showed some noteworthy differences in terms of geographical distribution and resistance traits. In particular, CG258, CG307 and ST101 had a broader geographical distribution compared with ST395 (Figure 1); each of the four lineages showed a specific and conserved pattern of antibiotic resistance-encoding genes (Figure 1 and Table S1). The antibiotic resistance profiles were similar for the antibiotics tested, with the exception of aminoglycosides and colistin. In CG258 isolates, amikacin resistance was much higher than gentamicin resistance; conversely, in CG307 and ST395 isolates, gentamicin resistance was higher than amikacin resistance, while in ST101 resistance was high to both aminoglycosides (Table 2). Colistin resistance was common among CG258 (43.2%), CG307 (48.4%) and ST101 (58.3%) isolates, but was not detected in ST395 (Table 2). Mutational events occurring in the *mgrB* locus represented the most frequent mechanism of colistin resistance (Table S1). The heterogeneous mutational events suggested that colistin resistance was not associated with clonal expansion, but rather with the local independent acquisition of several distinct mutations.

CG258 isolates

CG258 isolates exhibited the largest variability in core genome SNPs (SNP variation 0–1292; mean 187; median 63) if compared with isolates belonging to the other major lineages (Figure 2). SNP analysis identified 10 intralaboratory and three interlaboratory clusters of isolates (SNP variation 0–20) (Figure 2).

As for the capsular biosynthesis locus (KL), isolates belonging to ST512 and ST258 were consistently associated with KL107 (*wzi*-154) and KL106 (*wzi*-29), respectively, while in a previous study both KLs were identified in ST258.[7]

Concerning the resistome, the aminoglycoside resistance gene *aac(6′)-Ib* was detected in 92.3% of the isolates, and likely accounted for the high rate of amikacin resistance observed in this CG (Table S1).

The yersiniabactin-encoding locus (*ybt*) was detected in seven isolates, while the aerobactin locus (*iuc*) was found in a single isolate (Figure 1 and Table S1). These accessory loci for acquired siderophores are well-known genetic traits associated with increased virulence in *K. pneumoniae*.[24]

Table 1. Main characteristics (STs and carbapenemase genes) of 156 CR-KP isolates collected by 24 hospital laboratories participating in the AR-ISS located in different areas of Italy

Geographical area (number of isolates, %)	Laboratories[a]	Number of isolates	ST (number of isolates[b], type of carbapenemase)
North (50, 32%)			
	E	4	512 (2, KPC-3), 258 (KPC-2), 307 (KPC-2)
	F	3	512 (KPC-3), 23 (VIM-1), 253 (VIM-1)
	I	5	512 (4, KPC-3), 101 (NDM-1/OXA-48)
	L	4	307 (KPC-3; KPC-2), 17 (KPC-2), 3500 (KPC-2)
	M1	1	258 (KPC-2)
	M2	3	101 (KPC-2), 307 (KPC-3), 512 (KPC-3)
	M3	2	512 (2, KPC-3)
	Q	4	101 (4, KPC-2)
	S	16	512 (11, KPC-3), 14 (KPC-3), 15 (KPC-3), 307 (KPC-2), 395 (OXA-48), 3499 (KPC-3)
	T	3	307 (3, KPC-2)
	U	1	307 (KPC-3)
	V	4	101 (2, KPC-2), 15 (KPC-3), 512 (KPC-3)
Centre (48, 31%)			
	A	5	512 (2, KPC-3), 37 (KPC-3), 101 (KPC-3), 307 (KPC-3)
	B	6	512 (5, KPC-3), 2975 (KPC-3)
	J	19	512 (13, KPC-3), 101 (2, KPC-2), 395 (KPC-3), 1507 (KPC-3), 1519 (KPC-3), 3498 (KPC-3)
	P	16	307 (4, KPC-3; KPC-3/OXA-48), 512 (5, KPC-3), 101 (KPC-3; VIM-1), 147 (2, KPC-3), 15 (KPC-3), 45 (KPC-3)
	R	2	307 (2, KPC-3)
South (58, 37%)			
	C	1	307 (KPC-3)
	D	28	512 (11, KPC-3), 101 (9, KPC-3), 11 (3, KPC-3), 307 (3, KPC-3), 745 (KPC-3), 2185 (KPC-3)
	G	8	395 (5, KPC-3; OXA-48), 258 (2, KPC-2)
	H	1	101 (KPC-3)
	K	10	512 (8, KPC-3), 16 (KPC-3), 307 (KPC-3)
	N	9	307 (7, KPC-3; KPC-2), 512 (KPC-3)
	O	1	101 (KPC-3)

[a]For Laboratory codes, see legend to Figure 1.
[b]When the number of isolates is equal to 1, not reported.

CG307 isolates

CG307 isolates showed a lower core genome diversity (SNP variation 13–323; mean 99; median 83) compared with CG258 (Figure 2). Small clusters of isolates were identified, including four intralaboratory (SNP variation 13–20) and one interlaboratory (SNP variation 14–21) (Figure 2).

The aminoglycoside resistance gene *aac(3)-IIa* was present in 58.1% of the isolates, and likely accounted for the higher rate of gentamicin resistance observed in this CG (Table S1). All ST307 isolates carried the KL102 (*wzi*-173) locus. The *ybt* locus was detected in the majority of the isolates (70.9%, $n = 22$) (Figure 1 and Table S1).

ST101 isolates

Isolates belonging to ST101 exhibited a core genome diversity comparable to that of ST307 isolates (SNP variation 0–518; mean 95; median 64) (Figure 2). Two intralaboratory clusters of isolates and a small interlaboratory cluster were observed (SNP variation 0–21) (Figure 2).

All ST101 isolates were associated with the KL17 (*wzi*-137) locus. The 16S rRNA methylase ArmA, conferring a broad spectrum of resistance to aminoglycosides, was present in most isolates ($n = 19$; 79%). The *ybt* locus was detected in almost all ST101 isolates ($n = 23$; 95.8%) (Figure 1 and Table S1).

ST395 isolates

ST395 isolates showed a core genome diversity slightly higher than the other major lineages (SNP variation 10–974; mean 277; median 65). ST395 isolates were mainly collected from a single hospital laboratory, where a clonal expansion event was observed (SNP variation 0–23) (Figure 2). All ST395 isolates carried the KL2 (*wzi*-2) locus and the *iuc* and *ybt* loci (Figure 1 and Table S1).

Table 2. Antibiotic susceptibility profiles of 156 CR-KP isolates collected by hospital laboratories participating in the AR-ISS and of the four major lineages

	All isolates (%) (n = 156)			CG258 (%) (n = 74)			CG307 (%) (n = 31)			ST101 (%) (n = 24)			ST395 (%) (n = 8)		
	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
Meropenem	0.0	8.3	91.7	0.0	0.0	100.0	0.0	19.4	80.6	0.0	4.2	95.8	0.0	25.0	75.0
Imipenem	1.9	1.9	96.2	0.0	0.0	100.0	0.0	3.3	96.7	0.0	4.2	95.8	12.5	12.5	75.0
Ertapenem	0.0	–	100.0[a]	0.0	–	100.0	0.0	–	100.0	0.0	–	100.0[b]	0.0	–	100.0
Ceftazidime/ avibactam	97.4	–	2.6	100.0	–	0.0	100.0	–	0.0	91.7	–	8.3	100.0	–	0.0
Gentamicin	59.6	–	40.4	85.1	–	14.9	41.9	–	58.1	20.8	–	79.2	12.5	–	87.5
Amikacin	44.9	–	55.1	21.6	–	78.4	93.5	–	6.5	16.7	–	83.3	87.5	–	12.5
Ciprofloxacin	1.9	0.6	97.5	0.0	0.0	100.0	0.0	0.0	100.0	0.0	0.0	100.0	0.0	0.0	100.0
Trimethoprim/ sulfamethoxazole	24.4	3.8	71.8	16.2	1.4	82.4	6.5	3.2	90.3	58.3	12.5	29.2	50.0	12.5	37.5
Colistin	59.6	–	40.4	56.8	–	43.2	51.6	–	48.4	41.7	–	58.3	100.0	–	0.0
Chloramphenicol	16.0	–	84.0	1.4	–	98.6	45.2	–	54.8	8.3	–	91.7	0.0	–	100.0
Fosfomycin	82.7	–	17.3	74.3	–	25.7	93.5	–	6.5	87.5	–	12.5	87.5	–	12.5
Tigecycline	50.6	–	49.4	41.9	–	58.1	54.8	–	45.2	54.2	–	45.8	75.0	–	25.0

S, susceptible, standard dosing regimen; I, susceptible, increased exposure; R, resistant.
[a]Percentage calculated on 153 isolates, as for 3 isolates susceptibility testing yielded an MIC ≤1 mg/L that did not allow categorization of these isolates as S or R.
[b]Percentage calculated on 23 isolates, as for 1 isolate susceptibility testing yielded an MIC ≤1 mg/L.

Conclusions

To our knowledge, this study represents the first implementation of WGS for the surveillance of CR-KP in Italy, an action compliant with the high-priority recommendations of the ECDC for public health surveillances.[15,25] In this context, insights into the population structure, resistome and virulome of CR-KP circulating in Italy are provided, highlighting the emergence of high-risk lineages. The present findings suggest a significant evolution in the epidemiology of KPC-KP, from a predominance of the hyperepidemic CG258 towards a polyclonal population structure with the emergence of CG307 and ST101, as recently shown in some areas of the country.[3,7,11,26,27] Analysis of the population structure of the four major lineages identified clusters of isolates that suggested a local clonal expansion. We did not perform any outbreak investigation in the hospitals served by the laboratories; however, similar findings were previously reported in the EuSCAPE study[17] where isolates from the same hospital or from hospitals in the same country were generally found to be more closely related than isolates obtained in different countries.

Despite such an epidemiological shift, KPC production remains the most common mechanism of resistance to carbapenems, although it is no longer associated only with CG258, but also with three additional lineages, CG307, ST101 and ST395, that due to their genetic traits and the ability to disseminate can also be considered 'high-risk lineages'. Several recent studies indicated that ST307 is an emerging clone worldwide, with the ability to adapt to hospital settings, outcompeting CG258.[12,13,28,29] ST101 is an emerging clone at the global level,[26,30–32] while the distribution of ST395 appears still limited at present.[27,33]

The present data provide a snapshot of the evolution of CR-KP in Italy and can be considered as a baseline for future WGS-based surveys in Italy and on a wider scale.

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Members of the AR-ISS Laboratory Study Group on carbapenemase-producing Klebsiella pneumoniae

E. Manso, Ospedale Torrette Umberto I, Ancona; M. F. Pedna, Laboratorio Unico-Area Vasta Romagna, Cesena; M. Mungiguerra, Presidio Ospedaliero G. Moscati, Aversa (CE); A. Mosca, Università degli Studi di Bari, Facoltà di Medicina e Chirurgia, Dipart. DIM, Bari; F. Vailati, Azienda Ospedaliera Papa Giovanni XXIII, Bergamo; R. Aschbacher, Azienda Sanitaria dell'Alto Adige, Bolzano; A. Imbriani, Azienda Ospedaliero-Universitaria Policlinico Vittorio Emanuele, Catania; P. Sartore, Ospedale Civile di Cittadella (PD); C. Giraldi, Azienda Ospedaliera di Cosenza, Presidio Ospedaliero Annunziata, Cosenza; F. Piana, Azienda Sanitaria Ospedaliera S. Croce e Carle; P. Pecile, Ospedale Careggi, Florence; R. De Nittis, Azienda Mista Ospedaliera Universitaria, Foggia; B. Pini, Ospedale A. Manzoni, Lecco; P. Mirri, Azienda Ospedaliera Ospedale Civile di Legnano, Legnano (MI); E. Bianchi, Azienda Ospedaliera Ospedale di Circolo di Melegnano, Melegnano (MI); A. Restelli, Fondazione IRCCS Ca Granda Ospedale Maggiore Policlinico, Milan; D. Morelli, Istituto Tumori, Milan; M. R. Catania, Azienda Ospedaliero-Universitaria Federico II, Naples; A. Barbaro, Ospedali Riuniti Melacrino-Morelli, Reggio Calabria; P. Bernaschi, Ospedale Bambin Gesù, Rome; G. Parisi, Azienda Ospedaliera San Camillo-Forlanini, Rome; P. Gualdi, Ospedale S. Maria del Carmine, Rovereto (TN); P. A. Dusi, Ospedale di Sanremo, Sanremo (IM); R. Bona, Ospedale S. Paolo, Savona; M. M. D'Andrea, Università di Siena, Siena; R. Cavallo, Azienda Ospedaliero-Universitaria San Giovanni Battista, Turin; P. Lanzafame, Ospedale Santa Chiara, Trento; A. Sartor,

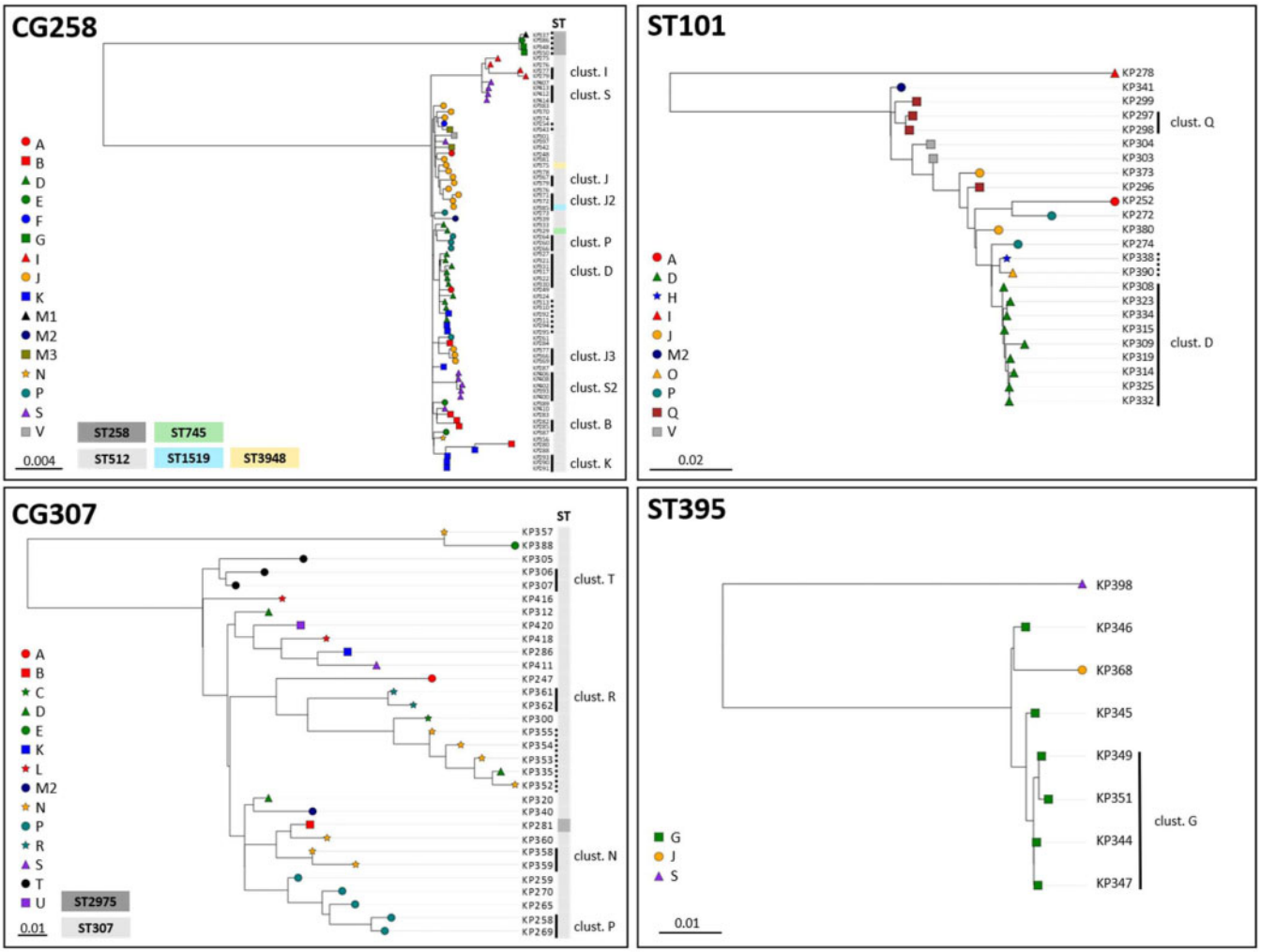

Figure 2. Phylogenetic relatedness within the four major represented CP-KP lineages. Core genome SNP-based phylogenetic trees of isolates belonging to: (i) CG258, including ST258, ST512, ST745, ST1519 and ST3498 (in different shades); (ii) CG307, including ST307 and ST2975; (iii) ST101; and (iv) ST395. For each phylogenetic tree, the hospital laboratories are indicated by labels of different shapes/colours. Laboratory codes are as in Figure 1. Isolates belonging to a cluster identified in a single laboratory are connected by a thick black line. Different clusters within the same laboratory are numbered progressively. Isolates belonging to a cluster identified in two or more laboratories are connected by a dotted black line. This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

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Transparency declarations

None to declare.

Supplementary data

Table S1 is available as Supplementary data at *JAC* Online.

References

1 Cassini A, Hogberg LD, Plachouras D *et al.* Attributable deaths and disability-adjusted life-years caused by infections with antibiotic-resistant bacteria in the EU and the European Economic Area in 2015: a population-level modelling analysis. *Lancet Infect Dis* 2019; **19**: 56–66.

2 Tumbarello M, Viale P, Viscoli C *et al.* Predictors of mortality in bloodstream infections caused by *Klebsiella pneumoniae* carbapenemase-producing *K. pneumoniae*: importance of combination therapy. *Clin Infect Dis* 2012; **55**: 943–50.

3 Giani T, Pini B, Arena F *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**: pii=20489.

4 Monaco M, Giani T, Raffone M *et al.* Colistin resistance superimposed to endemic carbapenem-resistant *Klebsiella pneumoniae*: a rapidly evolving problem in Italy, November 2013 to April 2014. *Euro Surveill* 2014; **19**: pii=20939.

5 Grundmann H, Glasner C, Albiger B *et al.* Occurrence of carbapenemase-producing *Klebsiella pneumoniae* and *Escherichia coli* in the European survey of carbapenemase-producing Enterobacteriaceae (EuSCAPE): a prospective, multinational study. *Lancet Infect Dis* 2017; **17**: 153–63.

6 ECDC. *Surveillance of Antimicrobial Resistance in Europe 2018*. Stockholm: ECDC, 2019.

7 Conte V, Monaco M, Giani T *et al.* Molecular epidemiology of KPC-producing *Klebsiella pneumoniae* from invasive infections in Italy: increasing diversity with predominance of the ST512 clade II sublineage. *J Antimicrob Chemother* 2016; **71**: 3386–91.

8 Wyres KL, Holt KE. *Klebsiella pneumoniae* population genomics and antimicrobial-resistant clones. *Trends Microbiol* 2016; **24**: 944–56.

9 Navon-Venezia S, Kondratyeva K, Carattoli A. *Klebsiella pneumoniae*: a major worldwide source and shuttle for antibiotic resistance. *FEMS Microbiol Rev* 2017; **41**: 252–75.

10 Wyres KL, Lam MMC, Holt KE. Population genomics of *Klebsiella pneumoniae*. *Nat Rev Microbiol* 2020; **18**: 344–59.

11 Bonura C, Giuffre M, Aleo A *et al.* An update of the evolving epidemic of bla_{KPC} carrying *Klebsiella pneumoniae* in Sicily, Italy, 2014. *PLoS One* 2015; **10**: e0132936.

12 Villa L, Feudi C, Fortini D *et al.* Diversity, virulence, and antimicrobial resistance of the KPC-producing *Klebsiella pneumoniae* ST307 clone. *Microb Genom* 2017; **3**: e000110.

13 Wyres KL, Hawkey J, Hetland MAK *et al.* Emergence and rapid global dissemination of CTX-M-15-associated *Klebsiella pneumoniae* strain ST307. *J Antimicrob Chemother* 2019; **74**: 577–81.

14 Bellino S, Iacchini S, Monaco M *et al. AR-ISS: sorveglianza dell'antibiotico-resistenza in Italia. Rapporto del quinquennio 2012–2016. Rapporti ISTISAN 18/22*. Rome: Istituto Superiore di Sanità, 2018.

15 ECDC. Expert Opinion on Whole Genome Sequencing for Public Health Surveillance. Stockholm: ECDC, 2016.

16 EUCAST. Breakpoint Tables for Interpretation of MICs and Zone Diameters. Version 10.0, 2020. http://www.eucast.org.

17 David S, Reuter S, Harris SR *et al.* Epidemic of carbapenem-resistant *Klebsiella pneumoniae* in Europe is driven by nosocomial spread. *Nat Microbiol* 2019; **4**: 1919–29.

18 Page AJ, Taylor B, Delaney AJ *et al.* SNP-sites: rapid efficient extraction of SNPs from multi-FASTA alignments. *Microb Genom* 2016; **2**: e000056.

19 Nguyen LT, Schmidt HA, von Haeseler A *et al.* IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Mol Biol Evol* 2015; **32**: 268–74.

20 Page AJ, Cummins CA, Hunt M *et al.* Roary: rapid large-scale prokaryote pan genome analysis. *Bioinformatics* 2015; **31**: 3691–3.

21 Chen L, Mathema B, Chavda KD *et al.* Carbapenemase-producing *Klebsiella pneumoniae*: molecular and genetic decoding. *Trends Microbiol* 2014; **22**: 686–96.

22 Drawz SM, Papp-Wallace KM, Bonomo RA. New β-lactamase inhibitors: a therapeutic renaissance in an MDR world. *Antimicrob Agents Chemother* 2014; **58**: 1835–46.

23 Albiger B, Glasner C, Struelens MJ *et al.* Carbapenemase-producing Enterobacteriaceae in Europe: assessment by national experts from 38 countries, May 2015. *Euro Surveill* 2015; **20**: pii=30062.

24 Lam MMC, Wyres KL, Judd LM *et al.* Tracking key virulence loci encoding aerobactin and salmochelin siderophore synthesis in *Klebsiella pneumoniae*. *Genome Med* 2018; **10**:77.

25 ECDC. ECDC Strategic Framework for the Integration of Molecular and Genomic Typing into European Surveillance and Multi-Country Outbreak Investigations—2019–2021. Stockholm: ECDC, 2019.

26 Del Franco M, Paone L, Novati R *et al.* Molecular epidemiology of carbapenem resistant Enterobacteriaceae in Valle d'Aosta region, Italy, shows the emergence of KPC-2 producing *Klebsiella pneumoniae* clonal complex 101 (ST101 and ST1789). *BMC Microbiol* 2015; **15**: 260.

27 Fasciana T, Gentile B, Aquilina M *et al.* Co-existence of virulence factors and antibiotic resistance in new *Klebsiella pneumoniae* clones emerging in south of Italy. *BMC Infect Dis* 2019; **19**: 928.

28 Castanheira M, Farrell SE, Wanger A *et al*. Rapid expansion of KPC-2-producing *Klebsiella pneumoniae* isolates in two Texas hospitals due to clonal spread of ST258 and ST307 lineages. *Microb Drug Resist* 2013; **19**: 295–7.

29 Ocampo AM, Chen L, Cienfuegos AV *et al.* A two-year surveillance in five Colombian tertiary care hospitals reveals high frequency of non-CG258 clones of carbapenem-resistant *Klebsiella pneumoniae* with distinct clinical characteristics. *Antimicrob Agents Chemother* 2016; **60**: 332–42.

30 Mammina C, Bonura C, Aleo A *et al.* Sequence type 101 (ST101) as the predominant carbapenem-non-susceptible *Klebsiella pneumoniae* clone in an acute general hospital in Italy. *Int J Antimicrob Agents* 2012; **39**: 543–5.

31 Kitchel B, Rasheed JK, Patel JB *et al.* Molecular epidemiology of KPC-producing *Klebsiella pneumoniae* isolates in the United States: clonal expansion of multilocus sequence type 258. *Antimicrob Agents Chemother* 2009; **53**: 3365–70.

32 Seki LM, Pereira PS, de Souza Mda P *et al.* Molecular epidemiology of KPC-2-producing *Klebsiella pneumoniae* isolates in Brazil: the predominance of sequence type 437. *Diagn Microbiol Infect Dis* 2011; **70**: 274–7.

33 Holt KE, Wertheim H, Zadoks RN *et al.* Genomic analysis of diversity, population structure, virulence, and antimicrobial resistance in *Klebsiella pneumoniae*, an urgent threat to public health. *Proc Natl Acad Sci USA* 2015; **112**: E3574–81.