

Importance of Surveillance of New Delhi Metallo-Beta-Lactamase *Klebsiella pneumoniae*: Molecular Characterization and Clonality of Strains Isolated in the Lazio Region, Italy

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Introduction: New Delhi metallo- β -lactamase producing *Klebsiella pneumoniae* (NDM-Kpn) strains have been causing healthcare-associated infections worldwide. The aim of this study was to describe the molecular mechanisms of antimicrobial resistance and to analyze the clonality of NDM-Kpn isolates collected between January 2019 and June 2020 from patients admitted to hospitals from the Lazio region, Italy.

Methods: We performed a retrospective cohort study. Whole-genome sequencing (WGS) was performed on all NDM-Kpn strains; clonality and genetic relationships were further investigated.

Results: During the surveillance period, 17 NDM-Kpn isolates were obtained from 17 patients admitted to seven different hospitals. Eight different sequence types (STs) were detected: ST147 (n = 4), ST383 (n = 4), ST15 (n = 3), ST11 (n = 2), ST17 (n = 1), ST29 (n = 1), ST307 (n = 1) and the newly identified ST4853 (n = 1). Genetic relationships were further investigated by the WGS-based core genome MLST (cgMLST) scheme, and 5 cluster types (CTs) were identified. Whereas a substantial overall heterogeneity among isolates was detected (8 different STs were identified out of 17 isolates), the strains within each cluster showed a very high level of genome similarity.

Discussion: Our study highlights the key role of surveillance, which allowed taking a picture of a part of the NDM-Kpn strains circulating in Italy, adding further insight into their molecular features.

Keywords: carbapenem-resistant *Klebsiella pneumoniae*, New Delhi metallo- β -lactamase, surveillance, healthcare-associated infections

Introduction

During the last decade, carbapenem-resistant *Enterobacterales* (CRE) have become endemic in many countries, representing a major health concern.¹ In *Enterobacterales* and particularly in *Klebsiella pneumoniae*, acquired Ambler class A (eg, KPC and GES enzymes), class B metallo-beta-lactamase (eg, VIM, IMP and NDM enzymes) and class D carbapenemases (eg, OXA-48-like enzymes) have been described.² Since its first isolation from a *K. pneumoniae* clinical isolate in 2008,³ an alarming spread of ceftazidime-avibactam (CZA) resistant NDM-producing *Klebsiella pneumoniae* isolates has been reported,¹ and these strains have been causing healthcare-associated infections worldwide, with an increase of cases across Europe in the last years.⁴

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Recently, an outbreak of NDM-producing *Enterobacteriales* occurred among patients without travel history hospitalized in the Tuscany region, Italy, with a total of 1645 cases of colonization or infection with NDM-CRE reported in the period from 1 November 2018 to 31 October 2019.^{5,6} Sporadic cases were also reported in four other Italian regions.⁶

Following the European Centre for Disease Prevention and Control (ECDC) Rapid Risk Assessment “Emergence of resistance to ceftazidime-avibactam in Enterobacteriaceae resistant to carbapenems”,⁷ the Italian Ministry of Health issued national recommendations for interventions, aimed at ensuring the identification and surveillance of CZA-resistant CRE. Since then, all these cases should be reported to regional surveillance services and isolates sent to reference laboratories. In addition, it is recommended to perform screening rectal swabs to all high-risk patients at hospitalization.

These recommendations are based on the principle that surveillance is the pillar to raise awareness of the emergence of CZA resistance and to limit its further spread. In the aforementioned ECDC risk assessment, a new European WGS-based surveillance module for extensively drug-resistant *Enterobacteriales* was launched with the aim of enabling the identification of mechanisms or gene mutations associated to CZA resistance and helping to monitor its geographic distribution among EU/EEA countries.

Importantly, data coming from deep molecular analysis of CZA-resistant *K. pneumoniae* strains circulating in Italy are still scarce. In particular, few data on NDM-Kpn strains are available, also due to their sporadic spread.

As regional reference center, we performed a retrospective cohort study with the aim of describing the molecular mechanisms and analyzing the clonality of all NDM-Kpn isolates collected from patients admitted in the Lazio region hospitals that were referred to our laboratory between January 2019 and June 2020.

Materials and Methods

We retrospectively included in the study all inpatients harboring a NDM-Kpn strain; for each patient, the following data were collected: age, gender, nationality, reporting hospital and ward, date of hospital admission, date and site of NDM-Kpn isolation.

For each NDM-Kpn isolate, species identification and antimicrobial susceptibility were obtained by MALDI-TOF MS (bioMérieux, Marcy l'Étoile, France)⁸ and Phoenix system (Becton Dickinson Diagnostics, CA, USA). Minimum inhibitory concentrations (MICs) for

carbapenems, aminoglycosides, trimethoprim/sulfamethoxazole, tigecycline and colistin were interpreted according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines.⁹ Phenotypic test for the identification of carbapenemase type was carried out by immunochromatographic assay (NG-test Carba, NG Biotech) that discriminated KPC, IMP, VIM, NDM, and OXA-48-like enzymes.

Whole-genome sequencing (WGS) was performed using Ion Torrent GSS5 (Life Technologies, Carlsbad, California, USA) by constructing single-end libraries with average lengths of 200 bp according to manufacturer's instructions. All raw reads generated were submitted to the Sequence Read Archive (SRA) under the BioProject accession number PRJNA686854.

Clonality was analysed by the traditional seven house-keeping genes multi-locus sequence type (MLST) extracted from the WGS data (<http://bigsd.b.pasteur.fr/klebsiella/klebsiella.html>). For an in-depth phylogenetic analysis, genetic relationships were further investigated by the validated WGS-based core genome MLST (cgMLST). Sequence quality trimming was carried out as described by Bletz et al,¹⁰ de novo assembly was performed by use of the Velvet assembler software (v1.1.04), integrated in the Ridom SeqSphere+ software (version 2.1, Ridom GmbH, Münster, Germany).¹¹ A total of 2358 target genes were used to characterize the gene-by-gene allelic profile of the *K. pneumoniae* strains, under the assumption that a well-defined cgMLST scheme should cover at least 95% of the genes present in all analysed isolates, compared to the reference strain (GenBank accession no. NZ_CP020067.1). The resulting set of target genes was then used for interpreting the clonal relationship displayed in a minimum spanning tree using the “pairwise ignore missing values” parameter during distance calculations. Genotypically related isolates (distance of ≤ 15 alleles) were identified within a Complex Type (CT) (<https://www.cgmlst.org>).

Antimicrobial resistance genes and plasmid replicons were extracted from the WGS data identified by in silico analysis using the ResFinder v3.0 web server (<http://www.genomicepidemiology.org>). The minimum percentage of sequence identity was set at 99%.

The data reported in this study come from the Regional Public Health Surveillance System and were recorded and analyzed in accordance with the recommendations on the surveillance of CZA-resistant CRE strains issued by the Italian Ministry of Health. All data were analyzed

anonymously; the research was carried out according to the principles set out in the Declaration of Helsinki. Informed consent was not required since this was a minimal risk retrospective study.

Results

During the surveillance period, 17 NDM-Kpn isolates were obtained from 17 patients admitted to seven different hospitals (H-1 to H-7), all located in the metropolitan area of Rome. Hospitals where stains were isolated, site of isolation and molecular characterization of isolates are reported in the Table 1. As shown, isolates were collected from different specimens; six out of 17 *K. pneumoniae* isolates were collected from rectal swabs; the remaining strains were from clinical samples, in particular, three were identified from blood cultures (Table 1).

Eight cases were reported by the same ward of hospital H-3 during a 6-month period; in four cases, the time between hospitalization and isolation of the NDM-Kpn strain was ≤ 24 hours. All patients hospitalized at H-3 originated from Libya and reported a recent hospitalization in North Africa or Turkey.

Three cases were reported from hospital H-4. Two of them were hospitalized in the same ward and during the same period; the time between hospitalization and isolation of the NDM-Kpn strains was >72 hours.

At hospital H-6 two cases were identified two months apart from each other; the time between hospitalization and isolation of the NDM-Kpn strains was for these cases >72 hours; a patient from H-6 had previously been admitted to a health-care facility in Tuscany region.

Hospitals H-1, H-2, H-5 and H-7 reported only 1 case each during the study period.

As expected, all NDM producing *K. pneumoniae* isolates were resistant to carbapenems. Antimicrobial susceptibility is shown in Table S1.

Immunochromatographic assay allowed the detection of NDM carbapenemase in all isolates; molecular analysis showed the presence of *bla*_{NDM-1} gene variant in 16/17 isolates and of *bla*_{NDM-5} gene variant in one isolate. Seven isolates additionally harbored OXA-48-like enzymes, 5/17 isolates the ampC cephalosporinase *bla*_{CMY-6} gene, 14/17 the ESBL *bla*_{CTX-M} gene and in only one isolate the presence of *bla*_{SCO-1} gene was documented (Table 1).

Eight different Sequence Types (STs) were identified: ST147 (n=4), ST383 (n=4), ST15 (n=3), ST11 (n=2), ST17 (n=1), ST29 (n=1), ST307 (n=1) and the newly identified ST4853 (n=1).

Clonal relationships within the STs using cgMLST scheme showed the presence of 5 Cluster Types (CTs). As shown in the minimum spanning tree obtained by Ridom pipeline, CT-1 was composed by 3 isolates, while CT-2, CT-3, CT-4 and CT-5 by 2 isolates each. Six strains did not cluster into any group (Figure 1).

The strains within each cluster showed a very high level of correlation (up to 11 allele differences). The isolates belonging to CT-1 and CT-5 originated from the same hospital (H-3); isolates belonging to CT-4 and CT-3 were also reported from the same hospital (H-6 and H-4, respectively), whereas isolates belonging to CT-2 were isolated from patients from two different hospitals (H-1 and H-2) (Figure 1).

Discussion

Our study describes the epidemiological and molecular features of NDM-Kpn strains circulating in the Lazio region, Italy. During an 18-month surveillance period, 17 patients harbouring NDM-Kpn strains were reported to our Center.

NDM-1 carbapenemase was predominant; in fact, only one strain harbored NDM-5. So far, 32 variants of NDM have been reported.¹²⁻¹⁵

According to the results of a systematic review on the worldwide spread and genotype distribution of human clinical isolates of NDM-Kpn based on published studies, the 4 most common variants of NDM carbapenemase in *K. pneumoniae* strains isolated from human samples are NDM-1, NDM-4, NDM-5 and NDM-7. In Europe, the common variants are *bla*_{NDM-1} and *bla*_{NDM-5}.¹⁶ Thus, our findings are consistent with available data on the distribution of NDM variants.

Additional enzymes, like OXA-48 and CMY, have been also identified in the majority of cases. Interestingly, 7 out of 17 isolates co-harboured *bla*_{NDM-1} and *bla*_{OXA-48} genes. The first co-producing OXA-48 and NDM-1 *K. pneumoniae* was identified in 2015 in Turkey.¹⁷ Recently, outbreaks due to these strains have been reported in Iran and Turkey.^{18,19} According to the results of a nationwide surveillance including 156 *K. pneumoniae* carbapenem-resistant strains from invasive infections provided from 24 laboratories located in different areas of Italy, in one isolate, *bla*_{OXA-48} gene was detected together with *bla*_{NDM-1} gene.²⁰

Of note, for one isolate, the presence of *bla*_{SCO-1} gene was documented, together with *bla*_{NDM-1}, *bla*_{SHV-155}, *bla*_{OXA-1}, *bla*_{TEM-1B} and *bla*_{CTX-M-15}. Since its discovery in 2007,²¹ the

Table 1 Molecular Analysis of NDM-Kpn Clinical Isolates

Isolate	Hospital ^a	Source	Genetic Determinants			Typing	
			Beta-Lactam	Additional Resistance Genes	Plasmid Replicon	ST ^b	Cluster ^c
NDM-Kpn-1	H-1	Rectal swab	<i>bla</i> _{NDM-1} , <i>bla</i> _{SHV-107}	<i>aac</i> (6)-Ib, <i>fosA</i> , <i>oqx</i> B, <i>sul</i> 1, <i>df</i> rA1/4	colRNAI, IncFIA (HI1)	11	2
NDM-Kpn-2	H-2	Rectal swab	<i>bla</i> _{NDM-1} , <i>bla</i> _{SHV-11}	<i>fosA</i> , <i>oqx</i> B, <i>sul</i> 1, <i>df</i> rA1/4	colRNAI, IncLM (pMU407)	11	2
NDM-Kpn-3	H-3	Wound swab	<i>bla</i> _{NDM-1} , <i>bla</i> _{OXA-48} , <i>bla</i> _{SHV28} , <i>bla</i> _{CMY-6} , <i>bla</i> _{CTX-M-15}	<i>aac</i> (6)-Ib3, <i>rmt</i> C, <i>fosA</i> , <i>oqx</i> A, <i>oqx</i> B, <i>sul</i> 2, <i>df</i> rA1/4	IncA/C2, IncLM (pOXA-48), IncR	15	1
NDM-Kpn-4	H-3	Wound swab	<i>bla</i> _{NDM-1} , <i>bla</i> _{OXA-9} , <i>bla</i> _{SHV-81} , <i>bla</i> _{TEM-1A} , <i>bla</i> _{CTX-M-15}	<i>aph</i> (3)-VI, <i>aac</i> (6)-Ib, <i>aac</i> (6)-Ib3, <i>aadA</i> 1, <i>armA</i> , <i>aac</i> (6)-Ib-cr, <i>fosA</i> _3, <i>fosA</i> _5, <i>msr</i> (E), <i>mph</i> (A), <i>cat</i> B3, <i>qnr</i> S1, <i>oqx</i> A, <i>oqx</i> B, <i>ARR-3</i> , <i>sul</i> 1, <i>sul</i> 2, <i>df</i> rA5	IncFIB(pQIL), IncR	147	-
NDM-Kpn-5	H-3	Wound swab	<i>bla</i> _{NDM-5} , <i>bla</i> _{OXA-9} , <i>bla</i> _{SHV-26} , <i>bla</i> _{TEM-1C} , <i>bla</i> _{CTX-M-15}	<i>aph</i> (3)-VI, <i>aac</i> (6)-Ib, <i>aadA</i> 1, <i>aph</i> (3)-Ia, <i>armA</i> , <i>fosA</i> _3, <i>fosA</i> _5, <i>msr</i> (E), <i>mph</i> (A), <i>mph</i> (E), <i>cat</i> A1, <i>qnr</i> S1, <i>oqx</i> A, <i>oqx</i> B, <i>sul</i> 1, <i>sul</i> 2, <i>test</i> (A), <i>df</i> rA5	IncFIB(Mar), IncHIIB	383	5
NDM-Kpn-6	H-3	Blood	<i>bla</i> _{NDM-1} , <i>bla</i> _{OXA-48} , <i>bla</i> _{SHV-55} , <i>bla</i> _{CMY-6} , <i>bla</i> _{CTX-M-15}	<i>aac</i> (6)-Ib3, <i>rmt</i> C, <i>fosA</i> , <i>oqx</i> A, <i>oqx</i> B, <i>sul</i> 1, <i>sul</i> 2, <i>df</i> rA1/4	IncA/C2, IncLM (pOXA-48), IncR	15	1
NDM-Kpn-7	H-4	Wound swab	<i>bla</i> _{NDM-1} , <i>bla</i> _{OXA-1} , <i>bla</i> _{OXA-10} , <i>bla</i> _{SHV28} , <i>bla</i> _{TEM-1B} , <i>bla</i> _{CTX-M-15}	<i>aph</i> (6)-Id, <i>aac</i> (6)-II, <i>aph</i> (3)-VI, <i>aac</i> (3)-Ile, <i>aac</i> (6)-Ib-cr, <i>aph</i> (3)-Ib, <i>fosA</i> _3, <i>cat</i> B3, <i>qnr</i> B1, <i>qnr</i> S1, <i>oqx</i> A, <i>oqx</i> B, <i>sul</i> 1, <i>sul</i> 2, <i>test</i> (A), <i>df</i> rA1/4	colRNAI, IncFIB(pQIL)	307	-
NDM-Kpn-8	H-3	Wound swab	<i>bla</i> _{NDM-1} , <i>bla</i> _{OXA-48} , <i>bla</i> _{SHV-106} , <i>bla</i> _{CMY-6} , <i>bla</i> _{CTX-M-15}	<i>aac</i> (6)-Ib3, <i>rmt</i> C, <i>fosA</i> , <i>oqx</i> A, <i>oqx</i> B, <i>sul</i> 2, <i>df</i> rA1/4	IncA/C2, IncLM (pOXA-48)	15	1
NDM-Kpn-9	H-3	Rectal swab	<i>bla</i> _{NDM-1} , <i>bla</i> _{OXA-48} , <i>bla</i> _{CMY-6} , <i>bla</i> _{SHV-148} , <i>bla</i> _{TEM-1B} , <i>bla</i> _{CTX-M-14b}	<i>aph</i> (6)-Id, <i>aac</i> (3)-IIa, <i>aac</i> (6)-Ib3, <i>aph</i> (3)-VIb, <i>aph</i> (3)-Ib, <i>rmt</i> C, <i>mph</i> (A), <i>fosA</i> _3, <i>cat</i> A1, <i>oqx</i> A, <i>oqx</i> B, <i>sul</i> 1, <i>test</i> (A)	IncA/C2, IncR, IncLM(pOXA-48)	4853	-
NDM-Kpn-10	H-3	Rectal swab	<i>bla</i> _{NDM-1} , <i>bla</i> _{OXA-48} , <i>bla</i> _{CMY-6} , <i>bla</i> _{SHV-148} , <i>bla</i> _{OXA-9} , <i>bla</i> _{TEM-1C} , <i>bla</i> _{CTX-M-15} , <i>bla</i> _{CTX-M-14b}	<i>aph</i> (6)-Id, <i>aac</i> (6)-Ib, <i>aph</i> (3)-VI, <i>rmt</i> C, <i>mph</i> (A), <i>aac</i> (3)-Ile, <i>aac</i> (6)-Ib-cr, <i>aph</i> (3)-Ib, <i>fosA</i> _3, <i>cat</i> A1, <i>qnr</i> S1, <i>oqx</i> A, <i>oqx</i> B, <i>sul</i> 1, <i>sul</i> 2, <i>test</i> (A)	IncA/C2, IncLM (pOXA-48)	383	5
NDM-Kpn-11	H-3	Wound swab	<i>bla</i> _{NDM-1} , <i>bla</i> _{SHV-155} , <i>bla</i> _{OXA-1} , <i>bla</i> _{TEM-1B} , <i>bla</i> _{CTX-M-15} , <i>bla</i> _{SCO-1}	<i>aph</i> (6)-Id, <i>aac</i> (3)-IIa, <i>aadA</i> 1, <i>aac</i> (6)-Ib-cr, <i>fosA</i> _3, <i>cat</i> B3, <i>qnr</i> B1, <i>oqx</i> A, <i>oqx</i> B, <i>sul</i> 1, <i>sul</i> 2, <i>test</i> (A), <i>df</i> rA15	IncFIB(pQIL)	17	-
NDM-Kpn-12	H-5	Blood	<i>bla</i> _{NDM-1} , <i>bla</i> _{OXA-1} , <i>bla</i> _{OXA-9} , <i>bla</i> _{SHV-11} , <i>bla</i> _{TEM-1A} , <i>bla</i> _{CTX-M-15}	<i>aac</i> (6)-Ib, <i>aph</i> (3)-VI, <i>aph</i> (3)-Ia, <i>aadA</i> 1, <i>armA</i> , <i>msr</i> (E), <i>mph</i> (A), <i>mph</i> (E), <i>aac</i> (6)-Ib-cr, <i>fosA</i> _5, <i>cat</i> B3, <i>qnr</i> S1, <i>oqx</i> A, <i>oqx</i> B, <i>ARR-3</i> , <i>sul</i> 1, <i>sul</i> 2, <i>df</i> rA5	colRNAI, IncFIB(pQIL), IncR	147	-
NDM-Kpn-13	H-6	Central venous catheter	<i>bla</i> _{NDM-1} , <i>bla</i> _{OXA-48} , <i>bla</i> _{SHV-148} , <i>bla</i> _{CTX-M-14b}	<i>aph</i> (6)-Id, <i>aph</i> (3)-Ib, <i>rmt</i> f, <i>mph</i> (A), <i>fosA</i> _3, <i>fosA</i> _5, <i>cat</i> A1, <i>qnr</i> B1, <i>oqx</i> A, <i>oqx</i> B, <i>AAR-2</i> , <i>test</i> (A)	IncFIB(pQIL), IncL	383	4
NDM-Kpn-14	H-4	Rectal swab	<i>bla</i> _{NDM-1} , <i>bla</i> _{OXA-1} , <i>bla</i> _{SHV-11} , <i>bla</i> _{TEM-1B} , <i>bla</i> _{CTX-M-15}	<i>aadA</i> 1, <i>aac</i> (6)-Ib-cr, <i>fosA</i> _3, <i>fosA</i> _5, <i>cat</i> B3, <i>qnr</i> B1, <i>oqx</i> A, <i>oqx</i> B, <i>sul</i> 1, <i>df</i> rA15	IncFIB(pQIL)	147	3

NDM-Kpn-15	H-6	Blood	<i>bla</i> _{NDM-1} , <i>bla</i> _{OXA-48} , <i>bla</i> _{SHV-148} , <i>bla</i> _{CTX-M-14b}	<i>aph(6)-Id</i> , <i>aph(3)-Ib</i> , <i>rmtF</i> , <i>mph(A)</i> , <i>fosa_3</i> , <i>fosa_5</i> , <i>catA1</i> , <i>qnrB1</i> , <i>oqxA</i> , <i>oqxB</i> , <i>AAR-2</i> , <i>tet(A)</i>	383	4
NDM-Kpn-16	H-4	Sputum	<i>bla</i> _{NDM-1} , <i>bla</i> _{OXA-1} , <i>bla</i> _{SHV-81} , <i>bla</i> _{TEM-1B} , <i>bla</i> _{CTX-M-15}	<i>addA1</i> , <i>aac(6)-Ib-cr</i> , <i>fosa_3</i> , <i>fosa_5</i> , <i>catB3</i> , <i>qnrB1</i> , <i>oqxA</i> , <i>oqxB</i> , <i>sul1</i> , <i>dftrA1_5</i> , <i>dftrA1_10</i>	147	3
NDM-Kpn-17	H-7	Rectal swab	<i>bla</i> _{NDM-1} , <i>bla</i> _{OXA-10} , <i>bla</i> _{SHV-2} , <i>bla</i> _{SHV-187} , <i>bla</i> _{TEM-1A}	<i>aac(6)-I</i> , <i>aph(3)-I</i> , <i>acc(3)-IId</i> , <i>aadA16</i> , <i>aac(6)-Ib-cr</i> , <i>fosa_3</i> , <i>floR_2</i> , <i>qnrB6</i> , <i>qnrS1</i> , <i>oqxA</i> , <i>oqxB</i> , <i>ARR-3</i> , <i>sul1</i> , <i>sul2</i> , <i>tet(A)</i> , <i>tet(D)</i> , <i>dftrAZ7</i>	29	-

Notes: ^aStrains collected from seven different hospitals in Rome (H-1 to H-7); ^bsequence type (ST) identified by MLST; ^ccluster types (CT-1 to CT-5) identified using cgMLST method. Abbreviations: CT, cluster type; H, hospital; NDM-Kpn, New Delhi metallo-β-lactamase producing *Klebsiella pneumoniae*; ST, sequence types; cgMLST, core genome multilocus sequence typing.

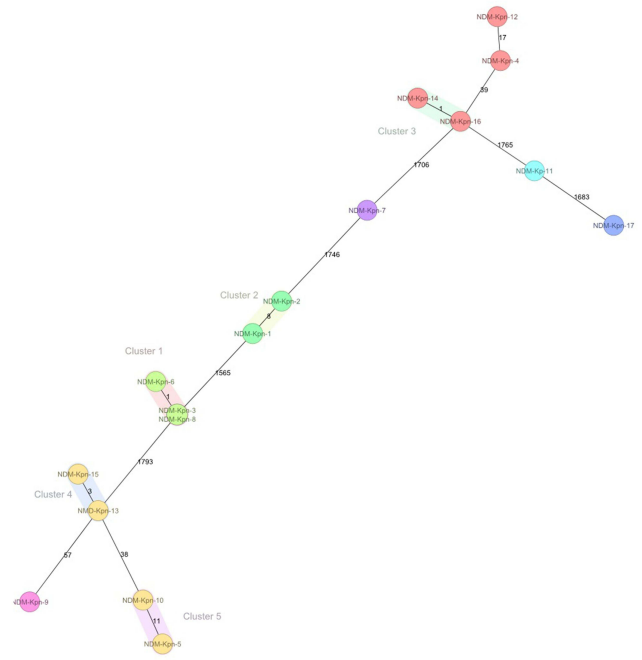


Figure 1 Minimum spanning tree based on cgMLST analysis of 17 NDM-producing *K. pneumoniae* isolates (NDM-Kpn-1 to NDM-Kpn-17), showing 5 cluster types numbered consecutively (1 and 5). Each circle represents an allelic profile, ie sequence type (ST), based on sequence analysis of up to 2358 target genes. Cluster types consist of closely related genotypes (≤ 15 allele differences). The numbers on the connecting lines illustrate the numbers of target genes with different alleles.

*bla*_{SCO-1} gene has been identified in few *K. pneumoniae* isolates and in other Gram-negatives.^{22,23} It should be emphasized that the real prevalence of this enzyme might be underestimated because it is not part of any routine screening for resistance genes. The importance of the finding of the *bla*_{SCO-1} gene in our small surveillance resides on its representativeness of the risk of transmission of uncommon plasmid-borne resistance genes. This finding further highlights the pillar role of molecular monitoring.

A substantial heterogeneity among isolates was detected; 17 strains belonged to 8 different STs and 5 CTs. Diverse range of STs is reported in different countries with a total of 86 unique STs reported globally for NDM-Kpn.¹⁶ In fact, in our study, the heterogeneity was lower among strains isolated from patients coming from the same geographic area. Analysis by cgMLST revealed that NDM-Kpn isolated from 5 patients at H-3 were clonally related; in particular, strains from patients #3, #6 and #8 belonged to ST15 (CT-1), whereas isolates from patient #10 and #5 belonged to ST383, being part of CT-5. ST15 and ST383 are reported in North Africa and Middle East.²⁴⁻²⁷ The NDM-Kpn strain isolated from patient #4,

who also originated from Lybia, harbored ST147, which has been reported in North Africa.^{28–30} In addition, a new ST (ST4853, an allelic variant of *phoE* gene, similar to ST383) was identified in our study, isolated from a Libyan patient hospitalized at H-3.

A higher heterogeneity was found among isolates from patients not reporting a recent hospitalization abroad; in fact, five different STs were documented (ST11, ST147, ST383, ST29 and ST307). In particular, in Italy ST307 has been recently identified as an emerging high-risk clone.^{31,32}

Interestingly, in two hospitals (H-6 and H-4) all strains isolated belonged to the same C-T, CT-4 and CT-3, respectively.

The main limitation of our study pertains the possible underestimation of the NDM-Kpn prevalence; we could not assess the level of hospitals' compliance in reporting patients harboring CZA-resistant Kpn as well as the total number of Kpn strains isolated in the investigated settings. Furthermore, we acknowledge the small sample size of our study; however, we believe that the surveillance data, even if they concern a few strains, are of paramount importance if representative of a specific geographic area.

Conclusion

Our findings are coherent with national data, reporting mainly sporadic cases of NDM-Kpn infections and colonizations.⁴ They represent a picture of part of the NDM-Kpn strains circulating in Italy, adding further insight into their molecular features. Our findings further emphasize the need for molecular surveillance that represents the basis of any strategy to struggle the spread of antimicrobial resistance.

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Disclosure

The authors report no conflicts of interest in this work.

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