

# Carbapenem non-susceptible *Klebsiella pneumoniae* from Micronet network hospitals, Italy, 2009 to 2012

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Carbapenem-resistant *Klebsiella pneumoniae* has recently been reported as a new, multidrug-resistant nosocomial pathogen in several hospitals from various Italian regions. Through Micronet, a new Italian sentinel laboratory-based surveillance network, we studied the trend of non-susceptibility of *K. pneumoniae* to selected carbapenems (imipenem and/or meropenem) in 14 of the 15 hospitals participating in the network. Analysis of data from 1 January 2009 to 30 April 2012 revealed a statistically significant increasing trend ( $p < 0.01$ ) in the proportion of carbapenem non-susceptible *K. pneumoniae* isolates from clinical specimens (from 2.2 % in 2009 to 19.4% in 2012). The increase in the proportion of non-susceptibility was very large for isolates from the respiratory tract (from 5.3% in 2009 to 38.5% in 2012) and blood (from 5.4% in 2009 to 29.2% in 2012). The results demonstrate the urgent need in Italy for infection control, guidelines, antibiotic stewardship programmes and utilisation of surveillance systems, such as Micronet, which are capable of receiving data from hospitals in real time for many pathogens and types of clinical specimens.

## Background

In recent years, carbapenem-resistant Enterobacteriaceae have emerged rapidly in hospitals worldwide [1]. Two main mechanisms can lead to reduced susceptibility or resistance to carbapenems in Enterobacteriaceae, namely reduced outer-membrane permeability associated with the production of extended-spectrum beta-lactamases (ESBLs) or AmpC-type beta-lactamases [2,3] and production of acquired beta-lactamases that degrade carbapenems (carbapenemases) [4,5]. The most frequent carbapenemases spreading among Enterobacteriaceae currently are the following: the KPC-type serine carbapenemases (belonging to Ambler's molecular class A); the VIM- and NDM-type metallo-beta-lactamases (belonging to Ambler's molecular class B); and the OXA-48-like

serine carbapenemases (belonging to Ambler's molecular class D) [6-9]. The species most commonly affected is *Klebsiella pneumoniae* [10-13]. Carbapenemase-producing Enterobacteriaceae (CPE) usually carry additional resistance determinants to other antimicrobial agents, making these strains resistant to many antibiotics [5] and thus leaving few therapeutic options for infected patients.

In Italy, carbapenem-resistant *K. pneumoniae* has recently been reported as a new, multidrug-resistant nosocomial pathogen in hospitals from different Italian regions [14-21]. The circulation of such strains in health-care facilities, however, needs to be clearly measured, since an increased circulation of carbapenem-resistant Enterobacteriaceae has important implications for both the control of both patient-to-patient transmission and hospital-to-hospital transfer of patients [22].

The purpose of this study is to assess the trend in the proportion of *K. pneumoniae* isolates non-susceptible to selected carbapenems from 2009 to 2012, in a sample of Italian hospitals belonging to Micronet. Micronet is a sentinel laboratory epidemiological surveillance network for infections, which has been established in Italy since 2008. It was created and is managed by the Istituto Superiore di Sanità, the Italian National Public Health Institute, and CINECA (a supercomputing centre and consortium of Italian universities). It is based on computerised daily collection of data on microbial isolates and of related antibiotic susceptibilities from the laboratory information systems of 27 laboratories nationwide.

## Methods

### Inclusion criteria

We collected data on *K. pneumoniae* isolates non-susceptible to selected carbapenems (imipenem

and/or meropenem) from relevant clinical specimens (bronchoalveolar lavages, tracheal aspirates, blood, cerebrospinal fluid, pus, urine) from 1 January 2009 to 30 April 2012 for 14 of the 15 hospitals in which the Micronet interface is fully active and functioning automatically (one hospital was excluded since it had no data for 2009). These are medium or large referral hospitals, located in four Italian regions. Since 2011, they have become part of ARISS, the Italian antimicrobial resistance surveillance system that sends data to the European Antimicrobial Resistance Surveillance Network (EARS-Net), coordinated by the European Centre for Disease Prevention and Control (ECDC).

### Micronet database

Data on *K. pneumoniae* isolates for which antimicrobial susceptibility testing results were available were extracted from the Micronet database, in which the results were described qualitatively (susceptible, intermediate, resistant). For the purposes of this study, isolates with intermediate and resistant profiles were defined as non-susceptible. We focused on the following subset of isolates (the data were obtained from the main dataset): isolates of *K. pneumoniae* from all major clinical specimens (blood, bronchoalveolar lavage, tracheal aspirate, cerebrospinal fluid, pus, urine) for which there were antimicrobial susceptibility test data available for the selected carbapenems (imipenem and/or meropenem). Information on susceptibility to ertapenem was not available for many of the participating laboratories for the entire period and was therefore not considered in our analysis.

For patients from whom several isolates had been obtained in the same month, only the first isolate was considered, regardless of the type of clinical specimen from which it was isolated and regardless the result. Multiple isolates from the same patient collected after an interval of 30 days were included. In such instances, we counted only the first isolate of *K. pneumoniae* for which susceptibility to the selected carbapenems was tested, regardless of the result.

An export procedure for the results of microbiological tests (both positive and negative) was developed in each participating laboratory, with the contribution of laboratory information systems managers and clinical microbiologists. Only results validated in the laboratory and used for clinical purposes are sent to a central server located and managed at CINECA, where the data are consolidated. We also used the Micronet database for an analysis stratified by type of clinical specimen.

### Additional data

The participating laboratories were also asked to provide full information on the methods used to identify the organisms (i.e. which automated system for identification was used) as well as which guidelines were used to interpret the results of the antimicrobial susceptibility tests. Furthermore, the laboratories were asked to provide the number of beds and the number

of patient days of their respective referral hospitals for January–December 2011, the most recent data available in all the hospitals.

### Data analysis

The proportion of carbapenem non-susceptibility was calculated as the number of carbapenem non-susceptible first isolates of *K. pneumoniae* divided by the total number of first isolates of *K. pneumoniae*, expressed as percentage. As not all participating laboratories had information on the age of the patients, age was not included in our analysis.

Epi-Info 3.53 [23] was used to calculate the proportion of isolates that were non-susceptible. OpenEpi 2.3.1 [24] was used to calculate confidence intervals for the proportion (using Fisher's exact test) and also for the extended Mantel–Haenszel chi-square test for linear trend.

### Results

The mean number of beds of the 14 Micronet hospitals in the study was 631 (median: 516.5; range: 322–1,220). The mean number of patient days was 183,388 (median: 155,084 (range: 84,360–372,646)).

Analysis of data from 1 January 2009 to 30 April 2012 from the 14 laboratories revealed a statistically significant increasing trend ( $p < 0.01$ ) in the proportion of *K. pneumoniae* isolates from clinical specimens that were non-susceptible to the selected carbapenems (Table 1). The percentage of non-susceptibility was higher for isolates from the respiratory tract, pus and blood.

The percentage of *K. pneumoniae* isolates non-susceptible to imipenem and/or meropenem was higher overall in isolates taken from patients in intensive care units and in medicine departments (Table 2).

Table 3 shows remarkable differences in the percentage of isolates non-susceptible to imipenem and/or meropenem among the 14 laboratories during the study period.

The automated systems used for susceptibility testing and the guidelines adopted by each laboratory are shown in Table 4. Until 2010, all laboratories had adopted the Clinical Laboratory Standards Institute (CLSI) interpretive criteria [25]. Before 2012, 13 of them moved to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) system [26] (Table 4).

### Discussion

In recent years, carbapenem-resistant *K. pneumoniae* has become a very important worldwide public health threat as a multidrug-resistant nosocomial pathogen [22]. Borer et al. have shown that the crude and attributable mortality rates (71.9% and 50%, respectively) associated with carbapenem-resistant *K. pneumoniae* bacteraemia were striking. More than 65% of patients with carbapenem-resistant *K. pneumoniae*

**TABLE 1**

*Klebsiella pneumoniae* isolates tested for susceptibility to imipenem and/or meropenem, by type of clinical specimen, 14 Micronet hospitals, Italy, 1 January 2009–30 April 2012 (n=11,353)

Clinical specimen	2009			2010			2011			2012 <sup>a</sup>		
	Total number of isolates	Number NS	% NS (95% CI)	Total number of isolates	Number NS	% NS (95% CI)	Total number of isolates	Number NS	% NS (95% CI)	Total number of isolates	Number NS	% NS (95% CI)
Respiratory sample <sup>b</sup>	226	12	5.3 (2.8–9.1)	331	89	26.9 (22.3–32.1)	396	159	40.2 (35.3–45.2)	91	35	38.5 (28.4–49.2)
Blood	166	9	5.4 (2.5–10.0)	283	64	22.6 (17.9–27.9)	344	112	32.6 (27.7–37.8)	89	26	29.2 (20.1–39.8)
Pus	164	5	3.0 (1.0–7.0)	253	33	13.0 (9.2–17.8)	307	90	29.3 (24.4–34.8)	75	27	36.0 (25.2–47.9)
Urine	2,282	37	1.6 (1.2–2.3)	2,774	153	5.5 (4.7–6.4)	2,794	279	10.0 (8.9–11.2)	766	110	14.4 (12.0–17.1)
Total <sup>c</sup>	2,840	63	2.2 (1.7–2.8)	3,646	341	9.4 (8.4–10.4)	3,846	642	16.7 (15.5–17.9)	1,021	198	19.4 (17.0–22.0)

NS: non-susceptible.

<sup>a</sup> 1 January–30 April.

<sup>b</sup> Bronchoalveolar lavages and tracheal aspirates.

<sup>c</sup> Includes 12 isolates from cerebrospinal fluid (2 in 2009, 5 in 2010, 5 in 2011, 0 in 2012).

**TABLE 2**

*Klebsiella pneumoniae* isolates tested for susceptibility to imipenem and/or meropenem, by type of hospital department from which specimens were obtained, 14 Micronet hospitals, Italy, 1 January 2009–30 April 2012 (n=11,301)<sup>a</sup>

Hospital department	2009			2010			2011			2012 <sup>b</sup>		
	Total number of isolates	Number NS	% NS (95% CI)	Total number of isolates	Number NS	% NS (95% CI)	Total number of isolates	Number NS	% NS (95% CI)	Total number of isolates	Number NS	% NS (95% CI)
Intensive care unit	254	18	7.1 (4.3–11.0)	352	85	24.1 (19.8–29.0)	444	187	42.1 (37.5–46.9)	103	42	40.8 (31.2–50.9)
Medicine	636	33	5.2 (3.7–7.3)	1,049	196	18.7 (16.4–21.2)	1,095	300	27.4 (24.8–30.2)	329	111	33.7 (28.7–39.2)
Surgery	284	4	1.4 (0.4–3.6)	393	35	8.9 (6.4–12.3)	367	65	17.7 (14.0–22.1)	102	15	14.7 (8.5–23.1)
Emergency room	53	2	3.8 (0.5–13.0)	84	8	9.5 (4.2–17.9)	100	18	18.0 (11.0–26.9)	32	3	9.4 (2.0–25.0)
Other	1,604	6	0.4 (0.2–0.9)	1,753	17	1.0 (0.6–1.6)	1,819	67	3.7 (2.9–4.7)	448	25	5.6 (3.7–8.2)

NS: non-susceptible.

<sup>a</sup> A total of 52 records did not contain any information on the hospital department and were excluded from the analysis.

<sup>b</sup> 1 January–30 April.

TABLE 3

*Klebsiella pneumoniae* isolates tested for susceptibility to imipenem and/or meropenem, by laboratory, 14 Micronet hospitals, Italy 1 January, 2009–30 April 2012 (n=11,353)

Laboratory	2009			2010			2011			2012 <sup>a</sup>		
	Total number of isolates	Number NS	% NS (95% CI)	Total number of isolates	Number NS	% NS (95% CI)	Total number of isolates	Number NS	% NS (95% CI)	Total number of isolates	Number NS	% NS (95% CI)
A	53	1	1.9 (0.0–10.1)	194	21	10.8 (6.8–16.1)	293	43	14.7 (10.8–19.3)	118	28	23.7 (16.4–32.4)
B	168	4	2.4 (0.7–6.0)	196	31	15.8 (11.0–21.7)	215	131	60.9 (54.1–67.5)	48	32	66.7 (51.6–79.6)
C	144	0	0.0 (0.0–2.5)	177	5	2.8 (0.9–6.5)	97	17	17.5 (10.6–26.6)	37	6	16.2 (6.2–32.0)
D	113	22	19.5 (12.6–28.0)	379	183	48.3 (43.2–53.4)	294	115	39.1 (33.5–45.0)	83	21	25.3 (16.4–36.0)
E	216	0	0.0 (0.0–1.7)	253	4	1.6 (0.4–4.0)	288	1	0.3 (0.0–1.9)	66	7	10.6 (4.2–53.4)
F	228	0	0.0 (0.0–1.6)	253	1	0.4 (0.0–2.2)	205	0	0.0 (0.0–1.8)	65	2	3.1 (4.2–53.4)
G	260	0	0.0 (0.0–1.4)	343	6	1.7 (0.7–4.0)	418	50	12 (9.1–15.6)	121	11	9.1 (4.6–15.7)
H	107	2	1.9 (0.2–6.6)	196	9	4.6 (2.1–8.5)	230	64	27.8 (22.1–34.1)	64	22	34.4 (22.9–47.3)
I	124	3	2.4 (0.5–3.9)	193	18	9.3 (5.6–14.3)	263	46	17.5 (13.1–22.6)	58	16	27.6 (16.7–40.9)
J	305	0	0.0 (0.0–1.6)	263	2	0.8 (0.1–2.7)	255	14	5.5 (3.0–9.0)	66	5	7.6 (2.5–16.8)
K	487	27	5.5 (3.8–8.1)	434	21	4.8 (3.1–7.4)	472	29	6.1 (4.2–8.8)	130	12	9.2 (4.9–15.6)
L	326	3	0.9 (0.2–2.9)	254	9	3.5 (1.6–6.6)	278	31	11.2 (7.7–15.5)	61	15	24.6 (14.5–37.3)
M	133	0	0.0 (0.0–2.7)	192	2	1.0 (0.1–3.7)	147	8	5.4 (2.4–10.4)	9	0	0.0 (0.0–33.6)
N	176	1	0.6 (0.0–3.1)	319	29	9.1 (6.3–12.9)	391	93	23.8 (19.7–28.4)	95	21	22.1 (14.2–31.8)

NS: non-susceptible.

<sup>a</sup> 1 January–30 April.

bacteraemia developed severe systemic inflammatory response syndrome and septic shock [27,28]. High rates of carbapenem non-susceptible *K. pneumoniae* have been seen in Greece since the early 2000s and in Cyprus since 2008, and a rapid increase has also been observed in Italy and Hungary since 2010 [29,30].

Experiences in single hospitals or entire countries have shown how the spread of carbapenem-resistant *K. pneumoniae* can be controlled by aggressive interventions of infection control, based on early identification of clinical infections and of colonised patients, to enforce in a timely manner stringent practices for the containment of the spread (isolation, hand hygiene, environmental cleaning and decontamination, etc.) [28]. Many institutions, including the United States Centers for Disease Control and Prevention, the United Kingdom Health Protection Agency and the European Centre for Disease Prevention and Control, have developed guidance to counter the spread of carbapenem-resistant Enterobacteriaceae, emphasising the importance of a prompt and effective response before the spread has reached such an extent that it cannot be controlled [6,22,29,31]. In Italy, there are no national guidelines for infection control, although at the local level, many hospitals have adopted guidelines and antibiotic stewardship programmes [32].

Data presented in this article from the Micronet network in Italy confirmed the notable increasing trend of non-susceptibility of *K. pneumoniae* to carbapenems since 2010, and revealed that the proportion that is non-susceptible has continued to increase in 2012. The proportions reported here are somewhat higher than those reported by EARS-NET for 2010 [30]: this could be explained by the large variability in the proportion of non-susceptible isolates among the laboratories and differences between the laboratories participating in the two networks. In fact, the percentage of *K. pneumoniae* non-susceptibility to carbapenems observed in 2010 by the Micronet network (Table 1) was highly affected by Laboratory D (Table 3); by eliminating data from that laboratory, the proportion of carbapenem non-susceptible *K. pneumoniae* isolates from blood observed in 2010 decreased from 22.6% to 10.5%. In 2011, however, data from Laboratory D had a lower impact due to the decreased proportion observed in that laboratory and to the increased proportions observed in the others: including data from Laboratory D, the proportions of non-susceptible isolates from all clinical specimens or blood were 16.7% or 32.6%, respectively, while, when data from this laboratory were not included, the proportions were 14.8% or 29.3%, respectively. These results underscore the possible impact of a large outbreak in a single hospital when aggregated or cumulative data are analysed.

The considerable heterogeneity observed in the proportion of carbapenem non-susceptible isolates in the different laboratories (Table 3) probably reflects the activities and organisation of the referral hospitals,

**TABLE 4**

Guidelines and automated systems for antimicrobial susceptibility testing used by laboratories in the 14 Micronet hospitals, Italy, 1 January, 2009–30 April 2012

Laboratory	Guidelines	Automated test system
A	Until September 2011: CLSI 2009 From October 2011: EUCAST	VITEK 2
B	Until June 2011: CLSI 2009 From July 2011: EUCAST	Phoenix, MicroScan WalkAway
C	Until June 2011: CLSI 2010 From July 2011: EUCAST	Phoenix
D	Until July 2011: CLSI 2011 From August 2011: EUCAST	Phoenix
E	Until June 2011: CLSI 2010 From July 2011: EUCAST	Phoenix
F	Until June 2011: CLSI 2010 From July 2011: EUCAST	MicroScan WalkAway
G	Until February 2011: CLSI 2009 From March 2011: EUCAST	VITEK 2
H	Until December 2010: CLSI 2010 From January 2011: EUCAST	Phoenix
I	Until December 2010: CLSI 2009 From January 2011: EUCAST	VITEK 2
J	Until June 2011: CLSI 2010 From July 2011: EUCAST	VITEK 2
K	Until May 2011: CLSI 2009 From June 2011: EUCAST	VITEK 2
L	Until December 2011: CLSI 2009 From January 2012: EUCAST	VITEK 2
M	Until May 2011: CLSI 2009 From June 2011: EUCAST	VITEK 2
N	Until April 2012: CLSI 2009	VITEK 2

CLSI: Clinical Laboratory Standards Institute; EUCAST: European Committee on Antimicrobial Susceptibility Testing.

their capacity for infection control and the geographical location of the hospital. For instance, Laboratory B, which reported 66.7% carbapenem non-susceptibility in 2012, is a multispecialised tertiary-level hospital with a transplant centre, which receives patients from other neighbouring hospitals or from other national hospitals. Differences in the use of various antibiotics/antibiotic classes in the hospitals could also be an important factor, as has been shown elsewhere in the scientific literature [33].

The higher proportion of non-susceptibility observed in isolates from respiratory samples, pus and blood versus those from urine might reflect, at least in part, the outpatient origin of the urinary isolates, with outpatients being less exposed to carbapenem-resistant Enterobacteriaceae circulating in the hospital environment. The Micronet system is able to differentiate between hospital departments but is unable to discriminate between samples from outpatients and inpatients. A system update will enable samples from inpatients and outpatients to be distinguished. It also

expected that patient characteristics, such as age, will be retrievable.

Changes in clinical breakpoints may also have influenced the proportion of isolates that were non-susceptible. The CLSI (formerly the National Committee for Clinical Laboratory Standards) in the United States modified its clinical breakpoints for carbapenems after an expert consultation in January 2010 [25]. They reduced the value of clinical breakpoints defined by minimum inhibitory concentrations for imipenem and meropenem from  $\leq 4$  to  $\leq 1$  mg/L for the category susceptible and from  $\geq 16$  to  $\geq 4$  mg/L for the category resistant. These thresholds were amended to better identify the carbapenemase-producing *K. pneumoniae*. The updated CLSI breakpoints came into use in June 2010. The clinical breakpoints adopted by EUCAST [26] in 2008 set their breakpoints for clinical purposes and not for optimal detection of carbapenemase production (they are one-dilution step higher than the modified CLSI value). Therefore some of the differences in the proportion of non-susceptible isolates could be a consequence of the changes in breakpoints [29].

The spread of carbapenem-resistant *K. pneumoniae*, and in particular of KPC-producing strains, is worrying from a public health point of view, since such strains are likely to be the source of many hospital-acquired infections in severely ill patients [5]. In addition, it is well known for its ability to accumulate and transfer resistance determinants, as illustrated with ESBLs [34]. Multidrug-resistant and pandrug-resistant KPC-producing bacteria may be the source of therapeutic failures, since novel anti-Gram-negative molecules are not expected in the near future [13]. For such reasons, there is an urgent need for infection control, antibiotic stewardship programmes and specific guidelines on strategies for screening for carriers.

In light of this analysis, we consider the development and use of networks, such as Micronet, in public health to be particularly important. Micronet is capable of receiving data from hospitals, collecting them in real time and detecting the emergence of these pathogens, which are particularly difficult to treat clinically and important to public health [29]. To strengthen the capacity of Micronet, in 2012, the results of antimicrobial susceptibility testing are being collected both quantitatively and qualitatively. Data are collected from the laboratory daily, from all types of clinical specimens. For such reasons, the network enables real-time monitoring of new microbiological alerts in multiple settings [35].

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