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**SURVEILLANCE** REPORT

# Carbapenem- and/or colistin-resistant *Klebsiella pneumoniae* in Greece: molecular follow-up survey 2022

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# Carbapenem- and/or colistin-resistant *Klebsiella pneumoniae* in Greece: molecular follow-up survey 2022


This report of the European Centre for Disease Prevention and Control (ECDC) was coordinated by Anke Kohlenberg and Marius Linkevicius.

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# Abbreviations

| | |
|---|---|
| CCRE survey | Survey of carbapenem- and/or colistin-resistant Enterobacterales |
| cgMLST | Core genome multilocus sequence typing |
| cgSNP | Core genome single-nucleotide polymorphisms |
| CPHL | Central Public Health Laboratory of Greece |
| ECDC | European Centre for Disease Prevention and Control |
| EEA | European Economic Area |
| ESBL | Extended spectrum beta-lactamase |
| EU | European Union |
| EUCAST | European Committee on Antimicrobial Susceptibility Testing |
| EuSCAPE | European survey of carbapenemase-producing Enterobacteriaceae |
| Mbp | Mega base pairs |
| NPHO | National Public Health Organization of Greece |
| NRL | National reference laboratory |
| OMP | Outer membrane protein |
| SNP | Single-nucleotide polymorphisms |
| SPAdes | St Petersburg genome assembler |
| ST | Sequence type |
| wgMLST | Whole genome multilocus sequence typing |
| WGS | Whole genome sequencing |

# Executive summary

The main aim of the genomic study described in this surveillance report was to determine the distribution of the highly drug-resistant clade of *Klebsiella pneumoniae* (sequence type (ST) 39 in Greek hospitals in 2022, after its rapid expansion which was documented in the survey of carbapenem- and/or colistin-resistant Enterobacterales (CCRE survey), coordinated by the European Centre for Disease Prevention and Control (ECDC) in 2019.

The continued circulation of this clade in 2022, was confirmed by the detection of *K. pneumoniae* ST39 isolates in 13 out of the 15 participating hospitals, even though the total number of isolates were found to be lower in 2022 (n=23) than in the same hospitals in 2019 (n=32). In addition, all the 15 participating hospitals had collected at least one isolate of this clade in 2019 and/or 2022, thereby highlighting that carbapenemase-producing *K. pneumoniae* ST39 can now be considered endemic in hospitals in Greece.

These findings are not only relevant due to the antimicrobial resistance profile of these isolates, but also as a marker of the rapid spread of a new, emerging antimicrobial resistance threat that has established itself in a hospital system; in this case, Greece. This situation is of further concern as a rapid spread may also occur for other antimicrobial-resistant pathogens (which are not a part of this study) with similar modes of transmission. Furthermore, the current dataset provides evidence that a similar event may have already occurred with *K. pneumoniae* ST323 carrying $bla_{KPC-2}$ which was not found in previous surveys in Greece – except for one carbapenem-susceptible isolate found in the European survey of carbapenemase-producing Enterobacteriaceae (EuSCAPE) coordinated by ECDC in 2013 – but was detected in six hospitals in 2022.

Based on the established genomic similarity cut-off, 44 within-hospital transmission events were detected in the 15 participating hospitals in the 2022 follow-up study. This is a high number considering that only up to 14 isolates per hospital were collected, which further indicates the ongoing transmission of carbapenemase-producing *K. pneumoniae* in the participating Greek hospitals. These transmission events mainly involved well-known international high-risk *K. pneumoniae* clones such as ST258/512 and ST11, as well as (based on current knowledge) the more Greece-specific high-risk *K. pneumoniae* clones, ST39 and ST323. The high number of within-hospital transmission events is likely one of the major causes of the rapid spread of newly emerging *K. pneumoniae* STs among the participating hospitals. However, the repeated transmission of high-risk *K. pneumoniae* clones to patients could be prevented with appropriate infection prevention and control measures if sufficient resources are made available.

To conclude, the high number of within-hospital transmission events is the most important finding of this study and needs to be urgently addressed as it constitutes a serious issue of patient safety in Greek hospitals. In addition, routine national molecular surveillance is essential to track emerging antimicrobial resistance threats, as well as to assess the effectiveness of the control efforts in use.

# 1 Background

In 2019, the European Centre for Disease Prevention and Control (ECDC) coordinated a structured survey of carbapenem- and/or colistin-resistant Enterobacterales (CCRE survey) and collected 3 035 *Klebsiella pneumoniae* isolates for whole genome sequencing (WGS)-based surveillance in 36 European countries. A preliminary analysis of the *K. pneumoniae* isolates from hospitals in Greece (n=257), which participated in the CCRE survey, showed the expansion of a highly antimicrobial-resistant clade of *K. pneumoniae* sequence type (ST) 39 carrying $bla_{KPC-2}$ and/or $bla_{VIM-1}$. This clade of *K. pneumoniae* ST39 was not present in the isolate collection from the European survey of carbapenemase-producing Enterobacterales (EuSCAPE) coordinated by ECDC in 2013 [1]. However, in 2019, it was detected in 12 of the 15 hospitals participating in the CCRE survey [2] throughout Greece. The extent of spread of this new clade in 2022 was not known and required further study.

## 1.1 Data collection and molecular analysis

A rapid follow-up survey was conducted to determine the prevalent STs of carbapenem-resistant *K. pneumoniae* in the same 15 Greek hospitals that participated in the CCRE survey (2019). The aim was to generate more timely data to inform public health action. The protocol, which was agreed on 6 July 2022, included isolate and data collection based on the CCRE survey protocol [2] with a few modifications to decrease the turn-around time from isolate collection to WGS results. In short, each of the 15 hospitals was invited to provide 10 consecutive isolates of *K. pneumoniae* that are in the categories of 'I' or 'R' for carbapenem susceptibility (i.e. 'susceptible, increased exposure' or 'resistant', based on the clinical breakpoints defined by the European Committee on Antimicrobial Susceptibility Testing – EUCAST) to the national reference laboratory (NRL) of Greece, i.e. the Central Public Health Laboratory (CPHL) of the National Public Health Organization (NPHO), together with a specific set of epidemiological and microbiological data. From the NRL, the isolates were forwarded to Eurofins Genomics (Konstanz, Germany) for WGS. Then, ECDC performed a preliminary analysis of the WGS results, including the data from EuSCAPE (2013), the CCRE survey (2019) and this follow-up study (2022).

In total, 502 *K. pneumoniae* isolates from Greece were analysed with 98 of these originating from EuSCAPE, 257 from the CCRE survey, and 147 from this follow-up study. WGS raw read data for 501 isolates were successfully assembled using SPAdes v3.15.3 and subjected to analysis in Pathogenwatch [3]. Out of these 501 isolates, 18 isolates did not pass quality control. Of these, 16 belonged to other species from the *K. pneumoniae* complex, i.e. *Klebsiella quasipneumoniae* (n=8), *Klebsiella variicola* (n=7), or were from other *Klebsiella* species, i.e. *Klebsiella oxytoca* (n=1). The two remaining isolates had genomes larger than 6.3 megabase pairs (Mbp). This resulted in 483 isolates being subjected to further analysis (comprising 97 isolates from EuSCAPE, 244 from the CCRE survey and 142 from this follow-up study). A phylogenetic tree was constructed based on core genome single-nucleotide polymorphisms (cgSNPs) using the neighbour-joining algorithm. Additionally, STs (Institut Pasteur scheme) [4], antibiotic resistance, and virulence profiles were determined using Kleborate v2.3.0 [5] within Pathogenwatch [6].

The dataset used for this analysis and report is available in Microreact.

# 2 Results

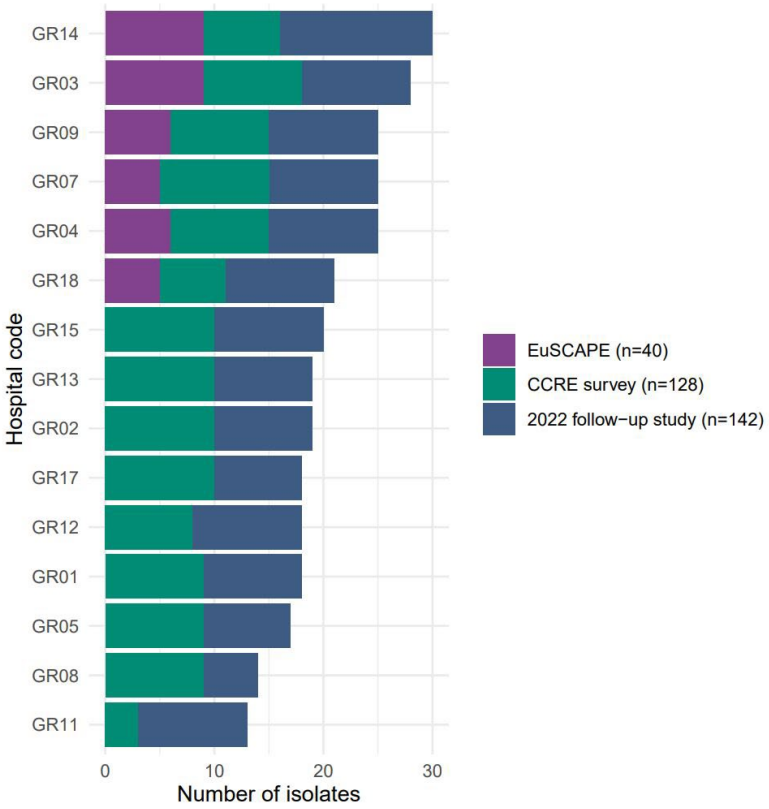
## 2.1 Number of isolates per participating Greek hospital

All the 15 invited hospitals participated in the 2022 follow-up study, and collected 5 to 14 carbapenem-I or R *K. pneumoniae* isolates. The total number of isolates included were 142 (Table 1). The first isolate was collected on 22 February 2022, and the last isolate on 3 October 2022. The protocol recommended the prospective collection of consecutive isolates to start after the finalisation of the protocol on 6 July 2022. However, 27 isolates had earlier collection dates between 22 February and 30 June 2022, indicating that stored isolates had also been retrospectively included by four hospitals. Table 1 presents the number of isolates collected in the 2022 follow-up study and the time of their collection. Figure 1 presents the distribution of carbapenem-R/I *K. pneumoniae* isolates for hospitals participating in all three studies, i.e. EuSCAPE (2013), CCRE survey (2019), and the follow-up study (2022).

**Table 1. Number of *Klebsiella pneumoniae* isolates by hospital and period covered (first to last isolate), Greek hospitals, 2022**

| Hospital identifier | Number of isolates in the 2022 follow-up study | Date of first isolate | Date of last isolate |
|---|---|---|---|
| GR01 | 9 | 04/08/2022 | 19/08/2022 |
| GR02 | 9 | 25/08/2022 | 06/09/2022 |
| GR03 | 10 | 03/09/2022 | 26/09/2022 |
| GR04 | 10 | 20/07/2022 | 21/08/2022 |
| GR05 | 8 | 02/09/2022 | 20/09/2022 |
| GR07 | 10 | 22/02/2022 | 18/08/2022 |
| GR08 | 5 | 26/07/2022 | 19/09/2022 |
| GR09 | 10 | 18/07/2022 | 26/07/2022 |
| GR11 | 10 | 13/06/2022 | 03/09/2022 |
| GR12 | 10 | 24/08/2022 | 07/09/2022 |
| GR13 | 9 | 26/07/2023 | 03/10/2023 |
| GR14 | 14 | 03/05/2022 | 10/08/2022 |
| GR15 | 10 | 17/03/2022 | 03/07/2022 |
| GR17 | 8 | 27/08/2022 | 09/09/2022 |
| GR18 | 10 | 24/08/2022 | 10/09/2022 |
| **Total** | **142** | **22/02/2022** | **03/10/2022** |

**Figure 1. Distribution of *Klebsiella pneumoniae* carbapenem-I or R isolates (n=310) in Greek hospitals, collected for EuSCAPE, the CCRE survey and the 2022 follow-up study**




*Four hospitals participating in the EuSCAPE study but without isolates in the most recent data collection were excluded from this figure.*

## 2.2. *Klebsiella pneumoniae* sequence type distribution in Greek hospitals

Overall, in the three studies, the most frequent ST among *K. pneumoniae* isolates was ST258/512, followed by ST11, ST39, ST147, and ST323. All the other STs were present with less than 10 isolates in the whole dataset. Of note, the lower number of 'Other STs' in the 2022 follow-up study is considered an 'artefact' due to its exclusive focus on carbapenem-I or R isolates, whereas the two previous studies included carbapenem-susceptible control isolates which resulted in a higher variety of STs. The distribution of carbapenem-I or R isolates by ST and their respective proportions in the dataset are shown in Table 2 (for all isolates) and Figure 2 (excluding EuSCAPE isolates which were partially collected in different hospitals), respectively. Three among the five most frequent *K. pneumoniae* STs in this dataset, i.e. ST258/512, ST11 and ST147, are international high-risk clones that also dominate among carbapenemase-producing isolates in other European Union (EU) Member States and worldwide [1,7], while the highly resistant clade of *K. pneumoniae* ST39 as well as *K. pneumoniae* ST323 seem to be locally circulating in Greece.

Whereas *K. pneumoniae* ST258/512 dominated in the previous studies in 2013 and 2019, *K. pneumoniae* ST11 has become the most frequent ST in the 2022 follow-up study. Due to their wide distribution and persistent detection for more than over 10 years, both *K. pneumoniae* ST258/512 and ST11 can be considered endemic in the participating Greek hospitals. Despite its recent emergence in Greece, the highly resistant clade of *K. pneumoniae* ST39 was detected in all the participating hospitals in the 2019 CCRE survey and/or the 2022 follow-up study. This indicates that this clade has also become established as a healthcare-associated pathogen in the participating Greek hospitals.

*K. pneumoniae* ST147 remained at similar proportion in the 2019 CCRE survey and the 2022 follow-up study. However, another *K. pneumoniae* ST, namely ST323, that was not found in 2019, appears to have rapidly spread between hospitals in 2022. This potentially repeats the rapid increase that was visible for ST39 in the CCRE survey (see Annex 1).

**Table 2. *Klebsiella pneumoniae* sequence type distribution for carbapenem-I or R isolates in EuSCAPE, the CCRE survey and the 2022 follow-up study in Greek hospitals (n=321)**

| Sequence type | Number of isolates (%) | | | |
|---|---|---|---|---|
| | EuSCAPE, 2013 | CCRE survey, 2019 | Follow-up study, 2022 | Total |
| ST258/512* | 27 (52.9) | 43 (33.6) | 41 (28.9) | 111 |
| ST11 | 13 (25.5) | 33 (25.8) | 47 (33.1) | 93 |
| ST39 | 1 (2.0) | 32 (25.0) | 23 (16.2) | 56 |
| ST147 | 1 (2.0) | 10 (7.8) | 10 (7.0) | 21 |
| ST323 | 0 (0.0) | 0 (0.0) | 13 (9.2) | 13 |
| Other STs | 9 (17.6) | 10 (7.8) | 8 (5.6) | 27 |
| **Total** | **51** | **128** | **142** | **321** |

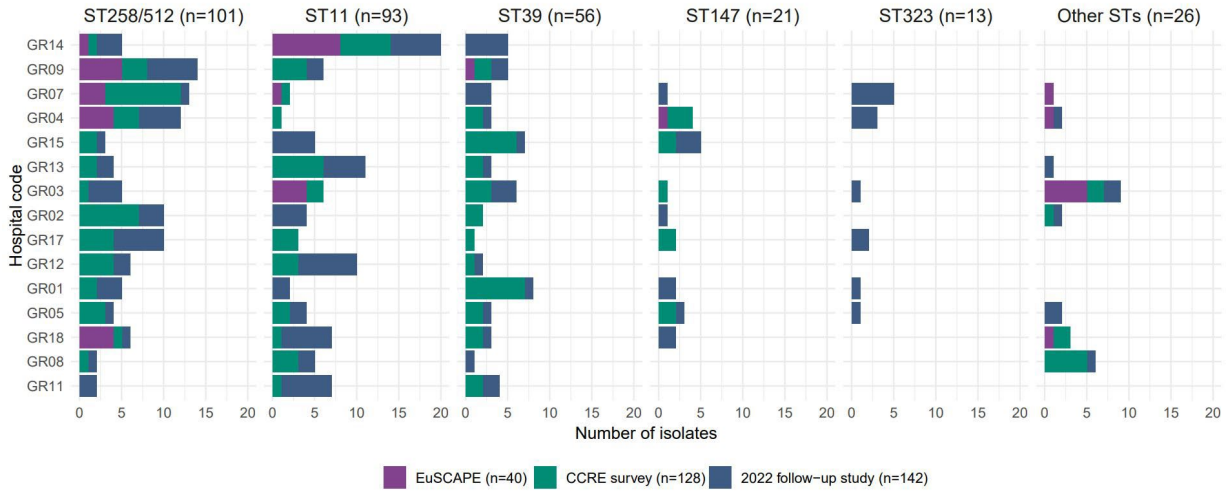
** This includes three isolates with single-locus variants of ST258.*

**Figure 2. Distribution of the five most frequent sequence types of carbapenem-I or R *Klebsiella pneumoniae* isolates in 15 Greek hospitals participating in the CCRE survey and the 2022 follow-up study (n=270)**


*ST: sequence type*

The three most frequent *K. pneumoniae* STs, i.e. ST258/512, ST11, and ST39 were detected in all the participating hospitals in the CCRE survey and/or the 2022 follow-up study. This highlights the wide distribution of these STs in Greek hospitals. The detection of *K. pneumoniae* ST147, ST323 and all 'Other STs' was more variable (Figure 3).

**Figure 3. Distribution of the five most frequent sequence types of *Klebsiella pneumoniae* carbapenem-I or R isolates (n=310) collected for EuSCAPE, the CCRE survey and the 2022 follow-up study in Greek hospitals**



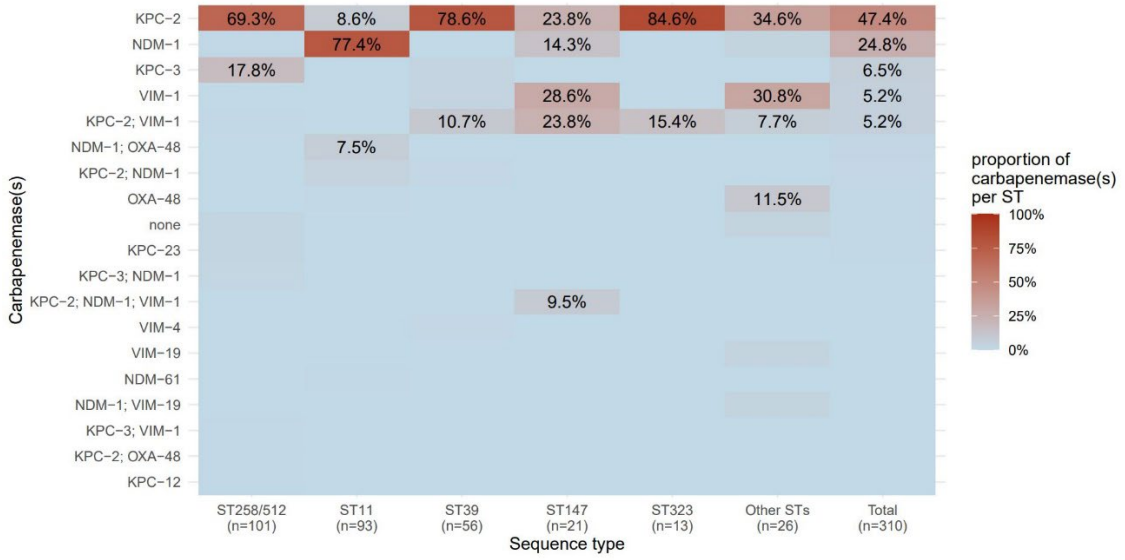

*ST: sequence type*

*Four hospitals that participated in the EuSCAPE survey but did not participate in the 2022 follow-up study were excluded. One* K. pneumoniae *ST323 isolate from EuSCAPE was detected in one of four excluded hospitals.*

## 2.3 Carbapenemase gene distribution for the five most frequent sequence types in the participating Greek hospitals

The most frequent carbapenemase gene detected in 179 carbapenem-I or R *K. pneumoniae* isolates was *bla*$_{KPC-2}$. *bla*$_{KPC-2}$ was the only carbapenemase gene detected in 155 isolates, and was present in various combinations with *bla*$_{NDM-1}$, *bla*$_{OXA-48}$, and/or *bla*$_{VIM-1}$ in the remaining 24 isolates. The distribution of carbapenemase genes was strongly associated with specific STs. The *K. pneumoniae* STs with the highest proportions of isolates carrying *bla*$_{KPC-2}$ in this dataset were ST323 (84.6%), ST39 (78.6%), and ST258/512 (70.3%) (Figure 4). The second most frequent carbapenemase gene in carbapenem-I or R isolates was *bla*$_{NDM-1}$ detected in 94 isolates, of which 77 only carried *bla*$_{NDM-1}$ and 17 showed various combinations with other carbapenemase genes. The *K. pneumoniae* ST with the highest proportion (77.4%) of isolates carrying *bla*$_{NDM-1}$ was ST11 (Figure 4).

**Figure 4. Distribution of carbapenemase genes in carbapenem-I or R *Klebsiella pneumoniae* isolates (n=310) among the five most frequent sequence types in EuSCAPE, the CCRE survey, and the 2022 follow-up study**

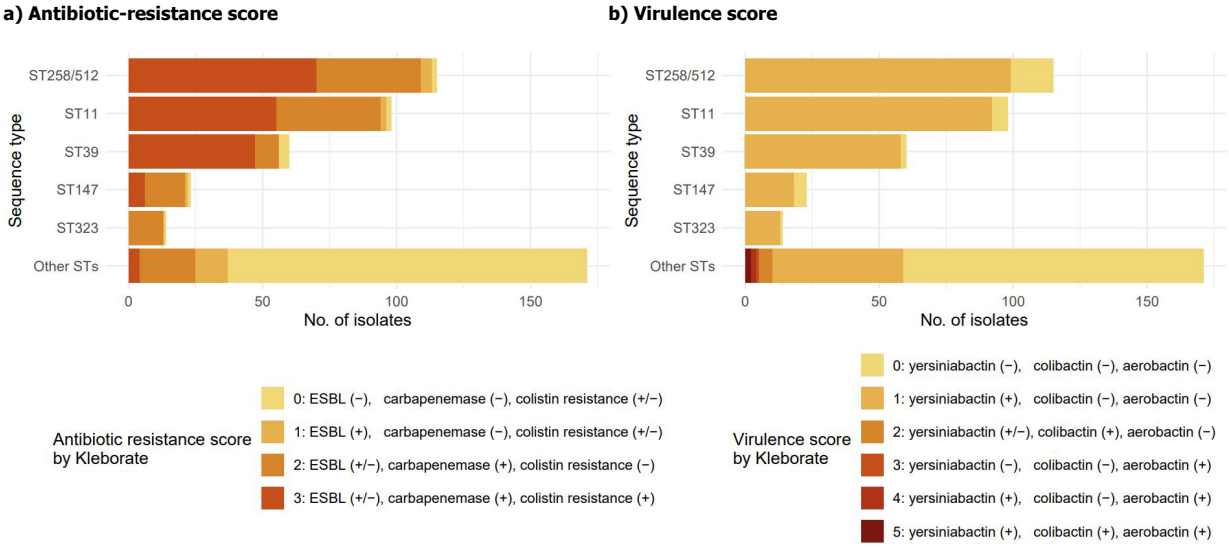



*ST: sequence type*
*Only percentages ≥5% are shown.*

An overview of the antibiotic-resistance and virulence scores of the isolates (including also the carbapenem-susceptible *K. pneumoniae* isolates from EuSCAPE and the CCRE survey) is displayed in Figures 5a and 5b, respectively. Figure 5a shows that the circulating high-risk clones not only acquired carbapenem resistance (resistance score 2), but also had a high level of predicted colistin resistance (resistance score 3). Most of the high-risk clones had a virulence score of 1 signifying the presence of yersiniabactin, but none had a higher virulence score.

Of note, two isolates in this dataset had the highest virulence score of 5. These two isolates were collected as part of the CCRE survey in 2019 and were carbapenem-susceptible isolates of the known hypervirulent *K. pneumoniae* ST23. These two isolates were detected in hospitals GR02 and GR14.

**Figure 5. Antibiotic-resistance and virulence score distribution among the five most frequent sequence types of *Klebsiella pneumoniae* isolates (n=483) from EuSCAPE, the CCRE survey and the 2022 follow-up study in Greek hospitals**

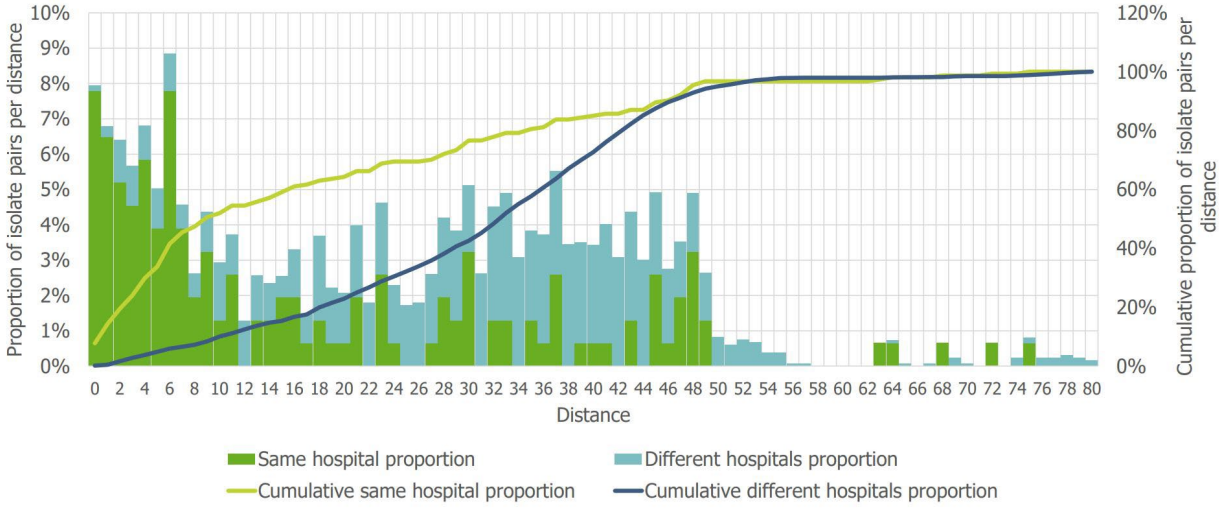



*ESBL: extended spectrum beta-lactamase; ST: sequence type*

## 2.4 Within-hospital transmissions

To estimate within-hospital transmissions, isolate pairs within the same *K. pneumoniae* ST from the 2022 follow-up study were used with an 80-SNP (single-nucleotide polymorphism) cut-off determined by Pathogenwatch. Each pair was annotated as 'same hospital' or 'different hospitals', based on the epidemiological information provided. For each isolate pair, distances were calculated using Pathogenwatch core genome SNPs, BioNumerics whole genome multilocus sequence typing (wgMLST) and core genome MLST (cgMLST) (scheme by Institut Pasteur). Histograms over distances for within- and between-hospitals isolate pairs were calculated for each method. Transitions in the slope of the cumulative proportions per distance as signs of a transition between phenomena were used as cluster cut-off candidates. In the Pathogenwatch comparison of within-/different-hospital isolate pairs, the optimum cut-off was determined to be in the range between 7 SNPs (45% of within-hospital isolate pairs and 6.6% of different-hospital isolate pairs included) and 11 SNPs (54% of within-hospital isolate pairs and 11% of different-hospital isolate pairs included) (Figure 6). To use a more conservative estimate, the cut-off was then set to ≤8 SNPs. Of note, the within-hospital isolate pairs from the 2022 follow-up study were very closely related not only in place (same hospital), but also in time due to the collection of consecutive carbapenem-I or R isolates in each hospital.

**Figure 6. Comparison of 'same hospital'-/'different hospitals' isolate pairs using Pathogenwatch core genome single-nucleotide polymorphisms (cgSNPs)**




Based on the above methodology, 44 within-hospital transmission events were detected in the 2022 follow-up study dataset with 12 (80%) out of the 15 participating hospitals having at least one within-hospital transmission event. Most transmission events occurred among the known high-risk *K. pneumoniae* clones, with most of the events related to ST11, which was involved in 22 (50%) of the 44 within-hospital transmission events, followed by ST258/512 and ST39 with seven events each (Table 3). The 12 hospitals with probable within-hospital transmission events were GR01 (n=1 transmission event), GR02 (n=3), GR03 (n=2), GR04 (n=1), GR07 (n=6), GR09 (n=3), GR11 (n=4), GR12 (n=5), GR14 (n=6), GR15 (n=5), GR17 (n=4), and GR18 (n=4). Details of these within-hospital transmission events are listed in Table 4.

**Table 3. Number of within-hospital transmission events by *Klebsiella pneumoniae* sequence type, detected in the 2022 follow-up study**

| *K. pneumoniae* sequence type | Number of within-hospital transmission events |
|---|---|
| ST11 | 22 |
| ST258/512 | 7 |
| ST39 | 7 |
| ST323 | 6 |
| ST147 | 2 |
| **Total** | **44** |

**Table 4. List of within-hospital transmission events of *Klebsiella pneumoniae* in the 2022 follow-up study**

| Hospital | Number of within-hospital transmission events | *K. pneumoniae* sequence type | Pathogenwatch distance between isolates in cgSNPs |
|---|---|---|---|
| GR01 | 1 | ST11 | 4 |
| GR02 | 3 (cluster) | ST11 | 1–6 |
| GR03 | 1 | ST39 | 0 |
| | 1 | ST258 | 2 |
| GR04 | 1 | ST323 | 0 |
| GR07 | 4 (cluster) | ST323 | 3–8 |
| | 2 (cluster) | ST39 | 2 and 4 |
| GR09 | 2 (cluster) | ST512 | 2 and 4 |
| | 1 | ST258 | 3 |
| GR11 | 4 (cluster) | ST11 | 0–4 |
| GR12 | 5 (cluster) | ST11 | 0–1 |
| GR14 | 4 (cluster) | ST39 | 1–5 |
| | 2 (cluster) | ST11 | 3–6 |
| GR15 | 2 (cluster) | ST147 | 2 each |
| | 3 (cluster) | ST11 | 1–4 |
| GR17 | 1 | ST323 | 0 |
| | 2 (cluster) | ST258 | 1 and 2 |
| | 1 | ST258 | 6 |
| GR18 | 4 (cluster) | ST11 | 0–7 |

*cgSNP: core genome single-nucleotide polymorphism*

# 3 Discussion

The main aim of this study was to determine the distribution of the highly drug-resistant clade of *K. pneumoniae* ST39 in Greek hospitals in 2022, after its rapid expansion which was documented in the CCRE survey in 2019. While there were a few previous case reports from Greece [8,9] or another country to which a patient from Greece was transferred [10], the extent of spread of this new clade only became evident in the systematic data collection from the CCRE survey.

This study confirmed the continued circulation of this clade in 2022, with the detection of *K. pneumoniae* ST39 isolates in 13 out of the 15 participating hospitals. However, the total number of isolates were lower in 2022 (n=23) than in the same hospitals in 2019 (n=32). In addition, all the 15 participating hospitals had collected at least one isolate of this clade in 2019 and/or 2022, thereby highlighting that carbapenemase-producing *K. pneumoniae* ST39 can now be considered endemic in hospitals in Greece. Of note, recent reports showed that *K. pneumoniae* ST39 carrying $bla_{KPC-2}$ developed resistance to ceftazidime-avibactam during treatment [9,10]. The spread of *K. pneumoniae* ST39 in Greece is not only relevant because of the highly drug-resistant profile of this new clade, but also as a marker of the rapid spread of a new, emerging antimicrobial-resistance threat which has established itself in a hospital system, in this case, Greece. The situation is of further concern as a rapid spread may also occur for other antimicrobial-resistant pathogens (which are not a part of this study) with similar modes of transmission.

The current dataset also provides evidence that a similar event may have already occurred with *K. pneumoniae* ST323 carrying $bla_{KPC-2}$. *K. pneumoniae* ST323 was not found in previous surveys in Greece (except for one carbapenem-susceptible isolate found in the EuSCAPE survey in 2013), but was detected in six hospitals in 2022. *K. pneumoniae* ST323 has been associated with the $bla_{CTX-M-15}$ extended-spectrum β-lactamase gene and healthcare-associated infections [11]. However, overall, it was an infrequent ST among *K. pneumoniae* isolates carrying carbapenemase genes in the CCRE survey (2019) with only two isolates of *K. pneumoniae* ST323 harbouring $bla_{KPC-3}$ that were detected in Italy. The spread of *K. pneumoniae* ST323 carrying $bla_{KPC-2}$ in Greek hospitals requires further follow-up.

Based on the established genomic similarity cut-off, 44 within-hospital transmission events were detected in the 15 participating hospitals in the 2022 follow-up study. This is a high number considering also that only up to 14 isolates per hospital were collected, and in most cases over a few weeks in each hospital. This is an indication of the ongoing transmission of carbapenemase-producing *K. pneumoniae* in the participating Greek hospitals. These transmission events mainly involved well-known international, high-risk *K. pneumoniae* clones such as ST258/512 and ST11, as well as (based on current knowledge) the more Greece-specific high-risk *K. pneumoniae* clones, ST39 and ST323. The high number of within-hospital transmission events is likely one of the major causes of the rapid spread of newly emerging *K. pneumoniae* STs among the participating hospitals. However, the repeated transmission of high-risk *K. pneumoniae* clones to patients could be prevented with appropriate infection prevention and control measures if sufficient resources are made available.

The high number of within-hospital transmission events is the most important finding of this study and needs to be urgently addressed as it constitutes a serious issue of patient safety in Greek hospitals. In addition, routine national molecular surveillance is essential to track emerging antimicrobial resistance threats, as well as to assess the effectiveness of the control efforts in use.

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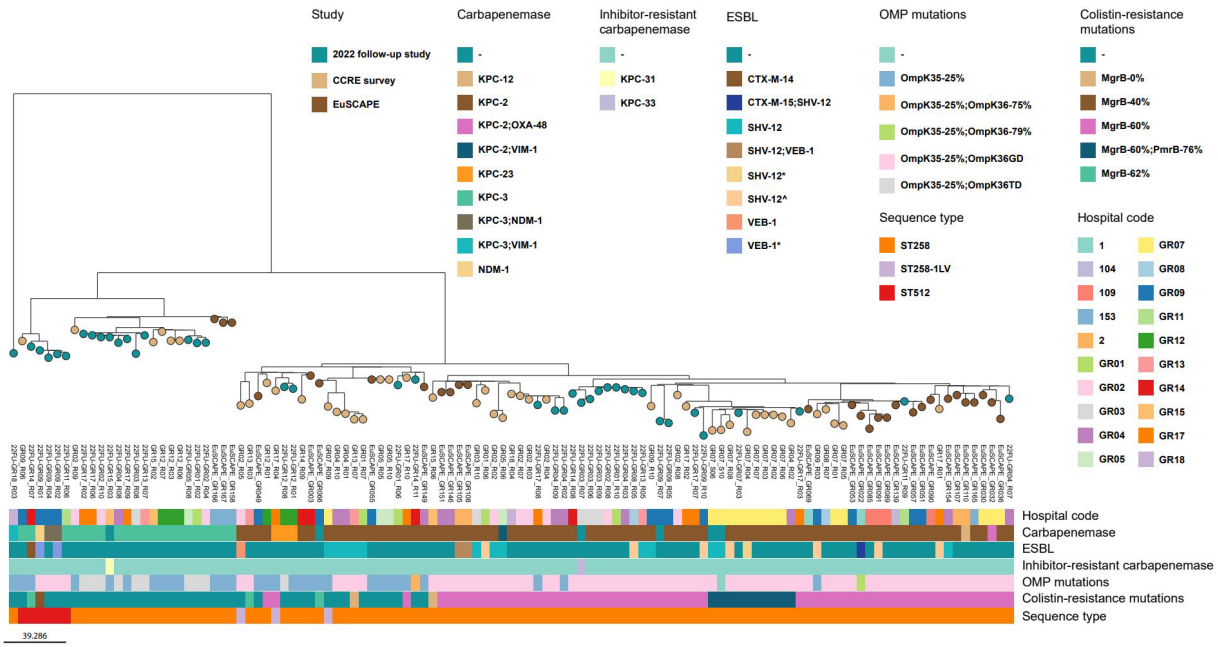
# Annex. Short description of specific *Klebsiella pneumoniae* sequence types (by order of decreasing frequency)

## *Klebsiella pneumoniae* ST258/512

*K. pneumoniae* ST258/512 (n=115 isolates) remains the most frequent ST in the overall dataset including carbapenem-susceptible and carbapenem-resistant isolates from all the three surveys – EuSCAPE (2013), CCRE (2019), and the follow-up study (2022). However, in the follow-up study, ST258/512 was the second most frequent ST after ST11. *K. pneumoniae* ST258/512 mainly carried $bla_{KPC-2}$ and less frequently $bla_{KPC-3}$. *K. pneumoniae* ST258/512 was the first high-risk ST to have spread in Greece [12] and accounted for >50% of carbapenem-I or R isolates in Greece as detected in the EuSCAPE survey (2013). Even though this proportion has now decreased to <30% of carbapenem-I or R isolates in the 2022 follow-up study, *K. pneumoniae* ST258/512 is still present in all the 15 participating hospitals with at least one isolate detected in 2022 (Figure A1).

**Figure A1. *Klebsiella pneumoniae* ST258/512 in EuSCAPE, the CCRE survey, and the 2022 follow-up study**



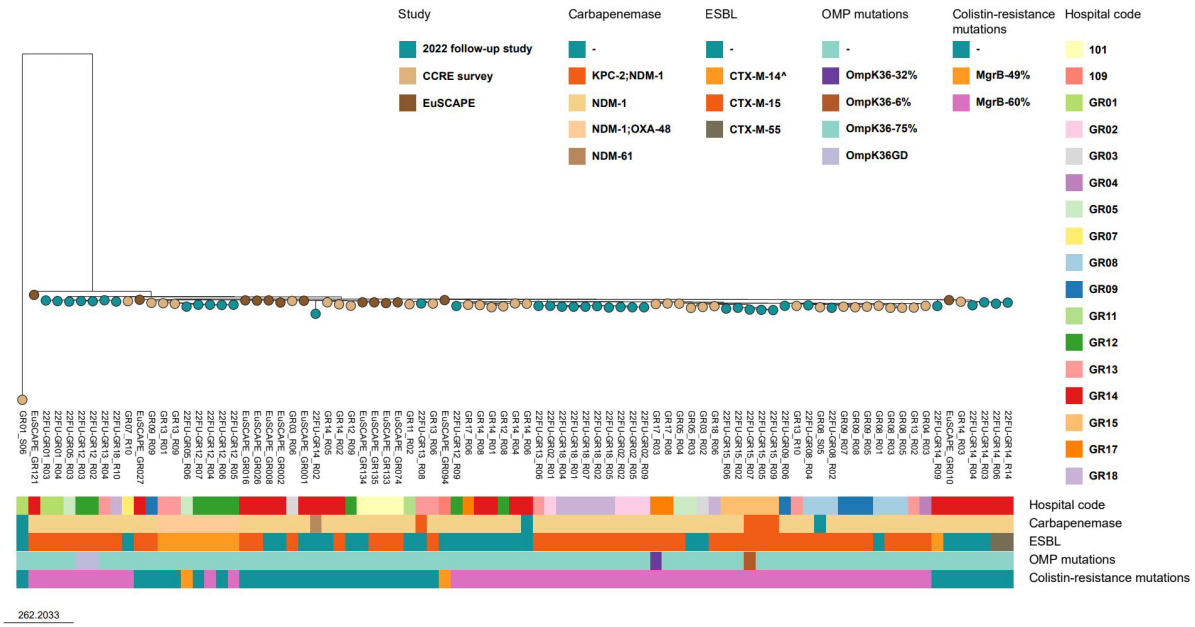

*ESBL: extended spectrum beta-lactamase; OMP: outer membrane protein*
** Inexact nucleotide and inexact amino acid match*
*^ Inexact nucleotide but exact amino acid match*

## *Klebsiella pneumoniae* ST11

The number of *K. pneumoniae* ST11 isolates detected in the overall dataset including carbapenem-susceptible and carbapenem-resistant isolates increased from 14 in the EuSCAPE survey (2013) to 38 in the CCRE survey (2019), and further to 47 isolates in the 2022 follow-up study (Figures A2 and A3). In 2022, *K. pneumoniae* ST11 was detected in 11 of the 15 participating hospitals. Only four hospitals (GR03, GR04, GR07, and GR17) did not detect *K. pneumoniae* ST11 isolates in 2022. In six hospitals, *K. pneumoniae* ST11 accounted for more than half of the collected carbapenem-I or R isolates.

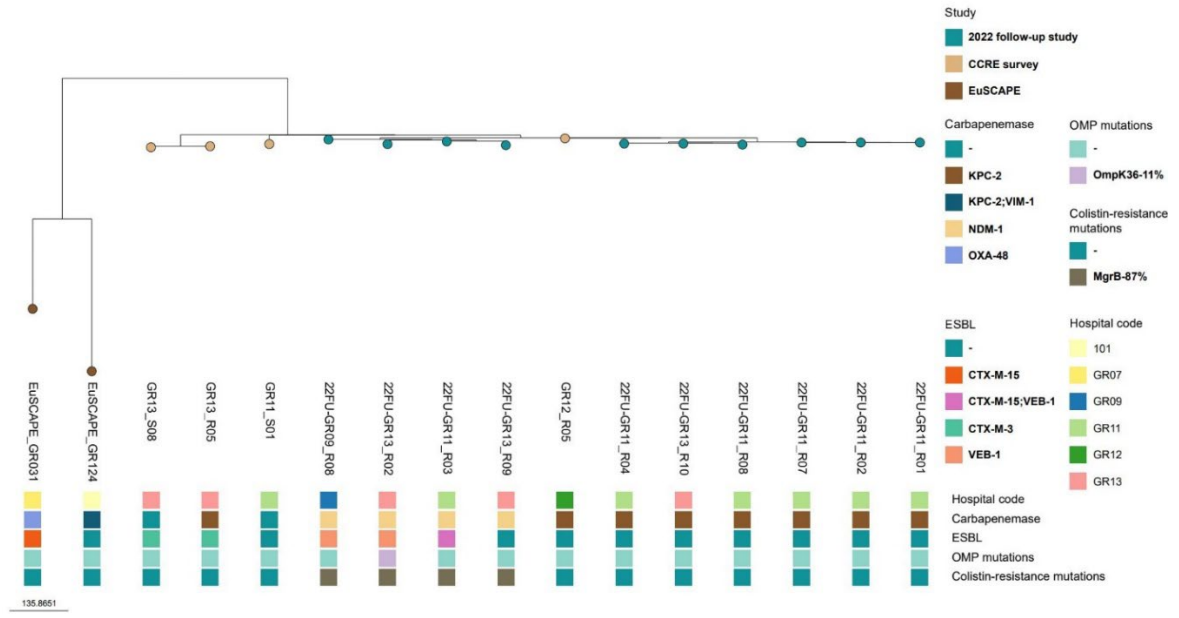
A possible explanation for the increase of *K. pneumoniae* ST11 in Greece might be its frequent association with $bla_{NDM-1}$ that confers resistance to ceftazidime-avibactam. The association between *K. pneumoniae* and $bla_{NDM-1}$ was observed for clade A (Figure A2), whereas $bla_{KPC-2}$ was the most frequently detected carbapenemase gene in isolates from clade B (Figure A3). Additionally, *K. pneumoniae* ST11 was also associated with half of the within-hospital transmission events in this dataset which highlights its adaptation for spread within hospitals.

**Figure A2. Clade A of *Klebsiella pneumoniae* ST11 in EuSCAPE, the CCRE survey, and the 2022 follow-up study**




*ESBL: extended spectrum beta-lactamase; OMP: outer membrane protein*
*^ Inexact nucleotide but exact amino acid match*

**Figure A3. Clade B of *Klebsiella pneumoniae* ST11 in EuSCAPE, the CCRE survey, and the 2022 follow-up study**




*ESBL: extended spectrum beta-lactamase; OMP: outer membrane protein*

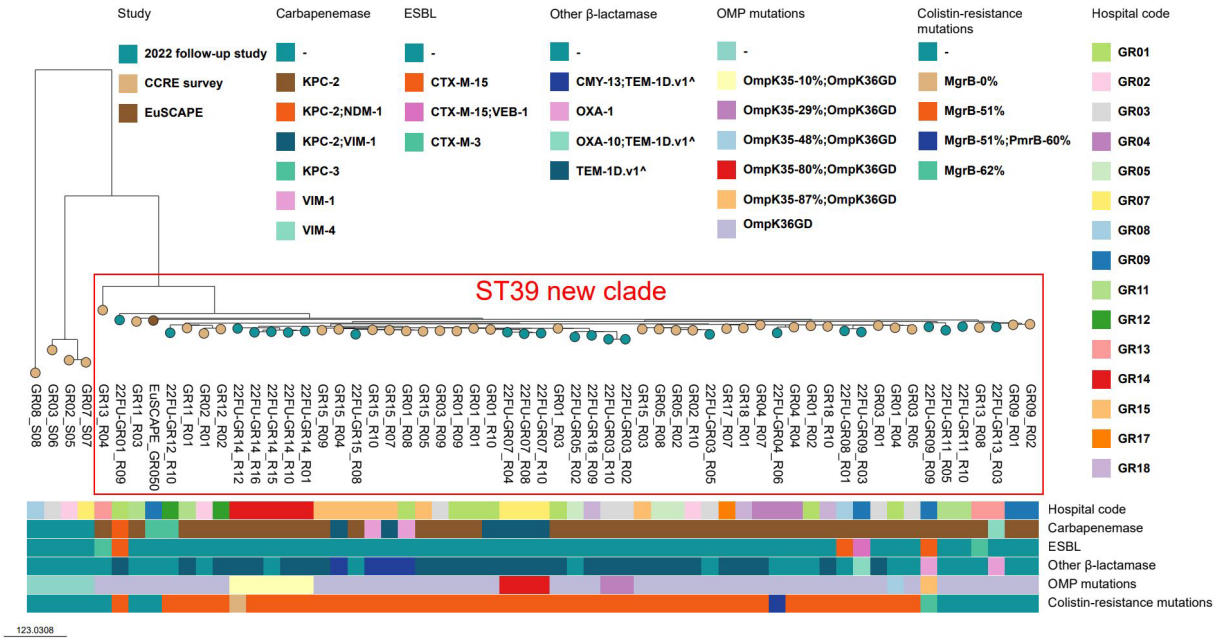
# *Klebsiella pneumoniae* ST39

The rapid expansion of the new clade of *K. pneumoniae* ST39 carrying $bla_{KPC-2}$ and/or $bla_{VIM-1}$ detected in the CCRE survey (2019) was the main reason to conduct this study. The results of the 2022 follow-up study show that this new clade continued to circulate in Greek hospitals, with 13 out of the 15 participating hospitals having collected at least one isolate belonging to the new clade (Table A1, Figure A4). Only two hospitals (GR02 and GR17) in which the new clade was detected during the CCRE survey (2019), did not find any *K. pneumoniae* ST39 isolates in 2022. However, three other hospitals (GR07, GR08, and GR14) in which the new clade was not present during the CCRE survey (2019), detected the new clade of *K. pneumoniae* ST39 in 2022. This indicates that the new clade of *K. pneumoniae* ST39 has established itself with continued circulation in the participating Greek hospitals.

**Table A1. Distribution of the new clade of *Klebsiella pneumoniae* ST39 in the participating hospitals in the CCRE survey and the 2022 follow-up study**

| Hospital | No. of *K. pneumoniae* ST39 isolates | |
|---|---|---|
| | CCRE survey, 2019 | Follow-up study, 2022 |
| GR01 | 7 | 1 |
| GR02 | 2 | 0 |
| GR03 | 3 | 3 |
| GR04 | 2 | 1 |
| GR05 | 2 | 1 |
| GR07 | 0 | 3 |
| GR08 | 0 | 1 |
| GR09 | 2 | 2 |
| GR11 | 2 | 2 |
| GR12 | 1 | 1 |
| GR13 | 2 | 1 |
| GR14 | 0 | 5 |
| GR15 | 6 | 1 |
| GR17 | 1 | 0 |
| GR18 | 2 | 1 |
| **Total** | **32** | **23** |

**Figure A4. *Klebsiella pneumoniae* ST39 expanding clade in EuSCAPE, the CCRE survey and the 2022 follow-up study**




*ESBL: extended spectrum beta-lactamase; OMP: outer membrane protein*
*^ Inexact nucleotide but exact amino acid match*

# *Klebsiella pneumoniae* ST323

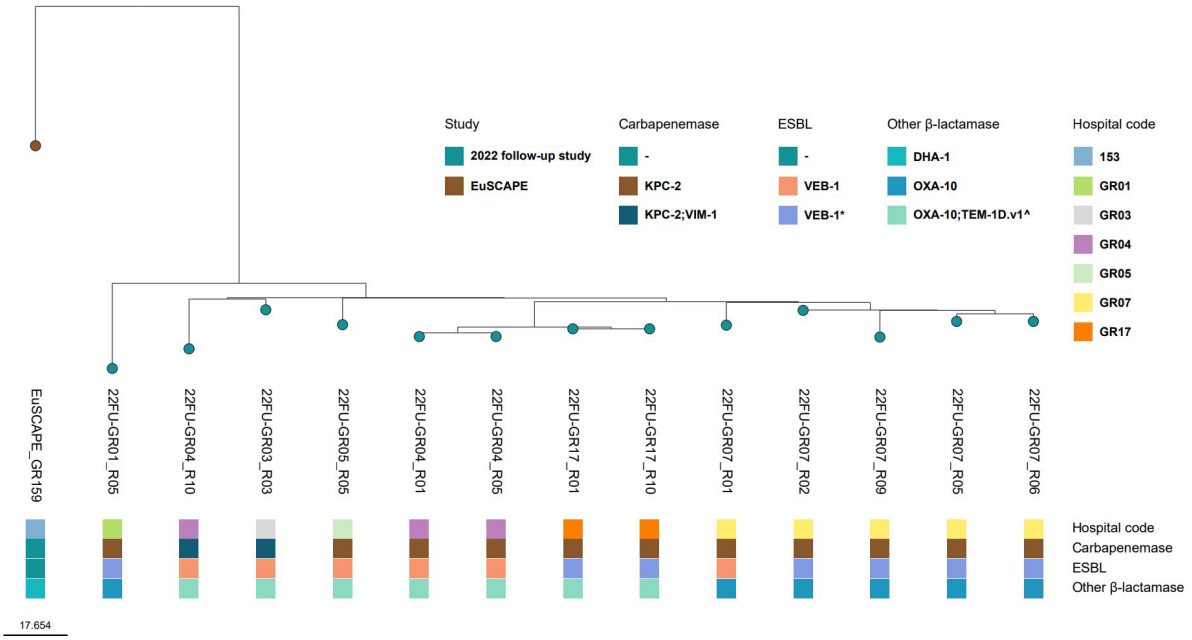
In addition to *K. pneumoniae* ST39, *K. pneumoniae* ST323 is another example of the emergence of a new *K. pneumoniae* ST with a rapid spread among Greek hospitals. While only one isolate of *K. pneumoniae* ST323 (carbapenem-susceptible) was found during the EuSCAPE survey (2013), none was found in the 15 participating hospitals in the CCRE survey (2019). However, 13 isolates of *K. pneumoniae* ST323 carrying $bla_{KPC-2}$ (with two isolates additionally carrying $bla_{VIM-1}$) collected in 2022 were present in six hospitals and accounted for six within-hospital transmission events (Table A2, Figure A5).

*K. pneumoniae* ST323 has been associated with carrying the $bla_{CTX-M-15}$ extended-spectrum β-lactamase gene and healthcare-associated infections [11]. However, overall, it was an infrequent ST among the *K. pneumoniae* isolates carrying carbapenemase genes in the CCRE survey (2019) with only two isolates of *K. pneumoniae* harbouring $bla_{KPC-3}$ that were detected in Italy. Three *K. pneumoniae* ST323 isolates carrying $bla_{KPC-2}$ had been previously reported from hospitals in Athens in a national survey conducted over the period from January 2009 to April 2010 [12].

**Table A2. Hospitals detecting at least one isolate of *Klebsiella pneumoniae* ST323 in the 2022 follow-up study**

| Hospital | No. of *K. pneumoniae* ST323 isolates | |
|---|---|---|
| | CCRE survey, 2019 | Follow-up study, 2022 |
| GR01 | 0 | 1 |
| GR03 | 0 | 1 |
| GR04 | 0 | 3 |
| GR05 | 0 | 1 |
| GR07 | 0 | 5 |
| GR17 | 0 | 2 |
| **Total** | **0** | **13** |

**Figure A5. *Klebsiella pneumoniae* ST323 in EuSCAPE, the CCRE survey, and the 2022 follow-up study**

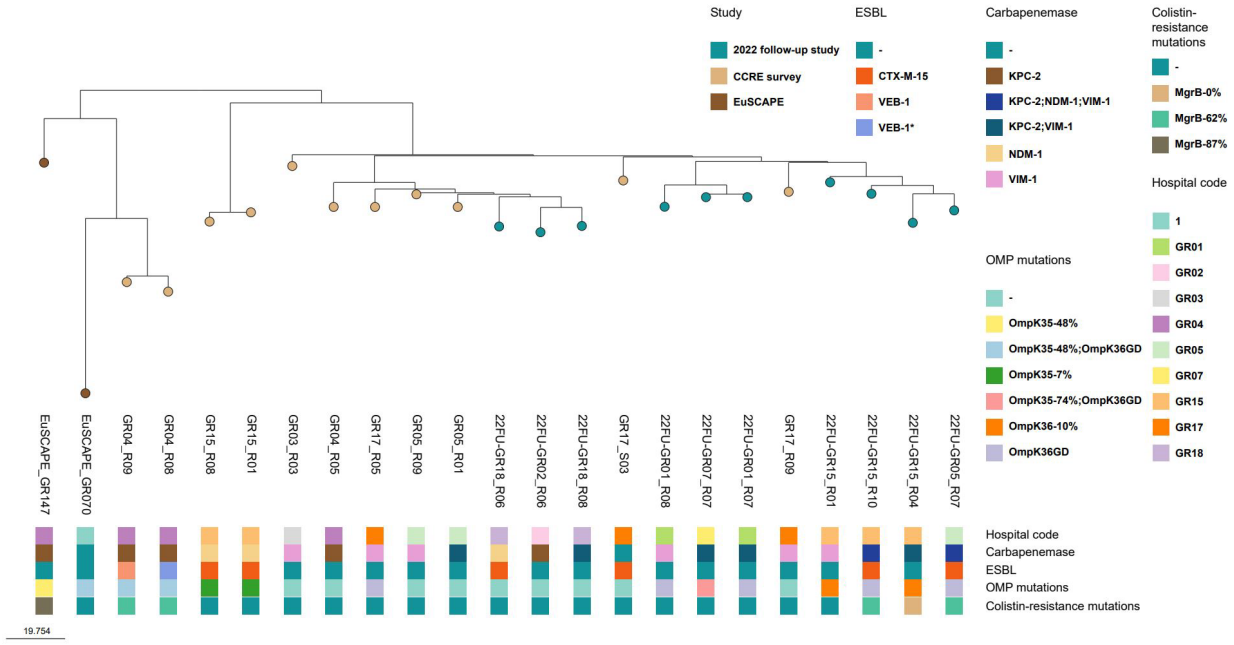



*ESBL: extended spectrum beta-lactamase*
** Inexact nucleotide and inexact amino acid match*
*^ Inexact nucleotide but exact amino acid match*

# *Klebsiella pneumoniae* ST147

*K. pneumoniae* ST147 is a well-known high-risk clone that has recently been involved in large outbreaks in EU Member States, for example, Italy [13]. However, in the 15 Greek hospitals which participated in this study, *K. pneumoniae* ST147 did not increase over time between the studies (CCRE, 2019 and follow-up study, 2022) and accounted for only two within-hospital transmission events.

**Figure A6. *Klebsiella pneumoniae* ST147 in EuSCAPE, the CCRE survey, and the 2022 follow-up study**




*ESBL: extended spectrum beta-lactamase; OMP: outer membrane protein*
** Inexact nucleotide and inexact amino acid match*

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