Antimicrobial Resistance and Infection Control

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Genomic surveillance reveals different transmission patterns between third-generation cephalosporin and carbapenem resistance in *Klebsiella pneumoniae* in the Comunidad Valenciana (Spain), 2018–2020

Neris García-González[1*], Beatriz Beamud[1,2], Jordi Sevilla-Fortuny[1], Victoria Sánchez-Hellín[3], Inmaculada Vidal[4], Juan Carlos Rodríguez[4], Begoña Fuster[5], Nuria Tormo[5], Carme Salvador[5], Concepción Gimeno[5,6], Barbara Gomila-Sard[7], Salvador Giner[8], Olalla Martínez[9], Javier Colomina[10], David Navarro[6,10,11], Victoria Domínguez[12], on behalf of Networked Laboratory for the Surveillance of Antimicrobial Resistance of the Valencian Community and Fernando González-Candelas[1,13*]

Abstract

Background The emergence and spread of third-generation cephalosporins (3GC) and carbapenem-resistant *Klebsiella pneumoniae* pose a global critical challenge. Understanding the transmission dynamics within and between hospital environments is crucial to develop effective control strategies.

Methods From 2017 to 2019, we conducted a genomic surveillance program in eight hospitals of the Comunitat Valenciana, Spain, collecting and sequencing 1,768 3GC- and carbapenem-resistant isolates. We quantified the overall transmission using core genomes and assessed the contribution of national and global isolates to the spread of AMR in the region by including 11,967 database genomes in the analysis.

Results The local collection was highly diverse, involving 188 lineages, including global high-risk clones such as ST307 and ST11, and 3GC and carbapenem resistance determinants. Half of the isolates were involved in transmission, with 70.5% occurring within hospitals.

Conclusions Different transmission patterns characterized the spread of 3GC- and carbapenem resistance in the region. While inter-hospital transmission played a significant role in the spread of 3GC-resistance, this was only sporadic for carbapenem resistance. Moreover, the factors behind inter-hospital spread for each type of resistance differed: while 3GC-resistance likely disseminated between hospitals through intermediate steps, carbapenem resistance was driven by more direct transmission routes. The burden of national and global cases on the ongoing regional AMR dissemination was low. Moreover, we revealed the rapid expansion in the region and globally

*Correspondence:
Neris García-González
neris.garcia@uv.es
Fernando González-Candelas
fernando.gonzalez@uv.es
Full list of author information is available at the end of the article

of lineage ST307 carrying the $bla_{CTX\text{-}M\text{-}15}$ gene, a main driver of local transmissions, providing a deeper understanding of the successful spread of this high-risk clone.

Keywords *Klebsiella pneumoniae*, Carbapenem resistance, Genomic surveillance

Introduction

Klebsiella pneumoniae is a notorious human pathogen, significantly contributing to the global burden of antimicrobial resistance (AMR) [1]. Specifically, third-generation cephalosporins (3GC)- and carbapenem-resistant *K. pneumoniae* are considered critical priority pathogens [2]. Among these, carbapenem resistance is particularly concerning as it threatens the last line of effective treatments [3].

Understanding the dynamics of AMR emergence and dissemination in hospital settings is essential for designing effective control strategies [1]. Previous studies have identified factors contributing to it, including imported cases from endemic regions [4], and community reservoirs [5, 6], as well as by horizontal transfer of plasmids from other species [7]. Recently it has been shown that non-clinical settings, such as farms or wastewaters, seem to have a limited impact on the spread of AMR to clinical settings [8, 9]. Within hospitals, transmission between patients has been shown to be the most important factor for spread [10–13]. However, the transmission dynamics between hospitals remains poorly understood. Most transmission studies focus on specific hospitals or wards (e.g. Intensive Care Units), or particular high-risk clones or resistant profiles [4, 6–8]. Thus, the relative contributions of hospitals, their surrounding communities, and global factors to the emergence and spread of 3GC- and carbapenem-resistant *K. pneumoniae* in clinical settings are yet unknown.

In this study, we aimed to elucidate the emergence and dissemination patterns of 3GC- and carbapenem-resistant *K. pneumoniae* strains in hospitals from the Comunitat Valenciana (CV), Spain, and to determine how national and global transmissions contributed to them. For this, we analyzed 1,768 genomes collected in the CV along with 11,967 global database genomes. Our results provide essential information to understand the AMR transmission dynamics in the region, ultimately contributing to the development of effective control measures to limit the emergence and spread of AMR.

Methods

Study design and sampling

From January 2017 to February 2020, we conducted a genomic surveillance study denominated Surveillance of *Klebsiella pneumoniae* in the Comunitat Valenciana (SKPCV), with the participation of eight reference hospitals that attend 45·5% of the region's population (~ 5 million inhabitants). The selection criteria were to include, for each hospital and trimester, the first 30 Extended-Spectrum β-lactamase (ESBL)- or carbapenemase-producing *K. pneumoniae* isolates, along with the first 10 3GC-susceptible clinical isolates. Only one isolate per patient was included. For each strain we collected basic demographic information about the patient and the antimicrobial susceptibility profile.

All isolates were sequenced using short-read technology, with Illumina NextSeq500. We selected a subset of isolates based on clinically relevant genetic backgrounds (e.g., belonging to high-risk clones or hypervirulent clades), AMR and virulence genes, and their combinations for further sequencing using PacBio or Nanopore long-reads technologies. Information on samples, accessions, and laboratory methods is provided in Supplementary Tables 1–2, Additional File 4.

Contextualization: additional retrospective local strains and database genomes

To contextualize the SKPCV collection locally, we included strains collected retrospectively for other projects in the same hospitals between 2010 to 2017. These isolates were collected and sequenced as the SKPCV isolates (Supplementary Table 1, Additional File 4).

For a global and national context, we included all *K. pneumoniae* genome assemblies from RefSeq (accessed on 6/13/2020), as well as all the read sets from the Sequence Read Archive (SRA) and the assemblies from the GenBank database labeled with "*Klebsiella pneumoniae*" and "Spain" (accessed on 4/4/2022) (Supplementary Table 2, Additional File 4).

The resulting collection was subjected to strict quality filters for taxonomy, assembly quality, and genome content. For details about bioinformatics, and statistical analyses see Supplementary methods, Additional File 1.

Definition of lineages and identification of transmissions

We used a 694-gene core genome MLST (cgMLST) scheme [14] to define lineages in the *K. pneumoniae* population. Chewbbaca [15] was used to cluster genomes into Clonal Groups (CGs) and sublineages (SLs) with the previously proposed cgMLST distance thresholds [16].

To investigate potential transmissions, we identified closely related clusters within SLs using the fastBAPS R

package v1.0.6 [17] and conducted a pangenome analysis for each cluster. To ensure accurate SNP calling from core genome alignments, we polished each gene alignment individually using a new pipeline. This allowed us to compare a much larger fraction of the genome than that obtained from cgMLST or reference mapping approaches. We defined transmission groups (TGs) as those isolates with pairwise SNP distances equal or fewer than 5 SNPs per Mbp compared [18]. For details regarding lineage definitions and TG analysis, see Supplementary methods. For detailed SNP thresholds by lineage see Supplementary Table 3, Additional File 4. All the code used can be found at https://github.com/NerisGarcia/Klebsiella_transmission.

Results

The SKPCV project

Under the SKPCV project, a total of 1,768 K. *pneumoniae* isolates were collected from eight hospitals (H1-H8) between January 2017 and February 2020 (Fig. 1A-C). Isolates were classified based on antimicrobial susceptibility testing (AST) or presence of 3GC- or carbapenem resistance genes (Supplementary results). As expected, due to the selection criteria, out of 1,604 isolates (90·7%) passing quality filters, 1,346 were resistant to 3GC (84%), 371 (23%) were also resistant to carbapenems, and 258 (16%) were sensitive to both (Fig. 1B). These represent 14·1%, 45·5%, and 0·009%, respectively, of the total diagnosed cases for those AMR patterns during the study period in the CV [19]. Isolates were mainly obtained from colonizations ($n = 870$) and infections ($n = 693$) (Supplementary Fig. 1, Additional File 3). The most frequent *K. pneumoniae* infection type was urine tract infections ($n = 192$, 27·7%) followed by bloodstream infections ($n = 149$, 21·5%).

3GC- and carbapenem-resistant population structure and determinants

The 1,604 genomes were grouped into 178 different cgMLST sublineages that corresponded to 188 STs (Fig. 2A and Supplementary Fig. 2, Additional File 3). The most frequently found STs were ST307 ($n = 559$), ST11 ($n = 147$), ST15 ($n = 91$), ST147 ($n = 87$), ST405 ($n = 62$), and ST219 ($n = 40$). 3GC-resistant strains ($n = 1,316$) were found in most STs (102 STs, 54·2%) whereas carbapenem-resistant strains ($n = 371$) were found in 38 STs (20·2%).

We used Fisher's exact test to determine if there were significant associations between lineages and resistance to 3GC or carbapenems (Fig. 2B). We found a higher-than-expected prevalence of 3GC-resistant strains in ST307, ST147, ST11, and ST405 ($p < 0·0001$). Conversely, ST14, ST45, ST37, ST17 and ST13 showed a significantly lower prevalence ($p < 0·0001$). For carbapenem resistance, we found a significant positive association with ST512, ST437, ST101, ST147, ST11 and ST392. In contrast, ST405, ST29, and ST219 showed lower prevalence of carbapenem-resistant isolates than other STs.

Next, we analyzed the genetic determinants conferring 3GC- and carbapenem resistance. 3GC-resistance was primarily driven by ESBL-coding genes, such as $bla_{\text{CTX-M}}$ ($n = 1187$, 74·0%) and bla_{SHV} ($n = 78$; 4·9%), and *AmpC* genes, such as bla_{DHA} ($n = 37$, 2·3%). $bla_{\text{CTX-M-15}}$ was the most prevalent determinant ($n = 981$, 61·1%) (Fig. 3A) and, despite being found in many clonal backgrounds ($n = 64$ STs), it was mainly associated with ST307 ($n = 532$, 54·5%; Fisher's exact test, $p < 0·0001$). This lineage (ST307-$bla_{\text{CTX-M-15}}$) represented 20·4% −45·3% of all isolates across hospitals. All the isolates carried at least one chromosomal bla_{SHV} allele, which is known to confer resistance to penicillins. Yet, we found that some isolates harbored a chromosomal $bla_{\text{SHV-12}}$ variant while others had acquired plasmid-mediated bla_{SHV} variants that conferred an extended resistance spectrum, including resistance to third generation cephalosporins.

Carbapenem resistance was mainly due to carbapenemases ($n = 326$, 87·8%), with $bla_{\text{OXA-48}}$ being the most prevalent gene ($n = 257$, 78·8%). We also found other carbapenemases, such as bla_{VIM} ($n = 37$, 11·3%) and $bla_{\text{KPC-3}}$ genes ($n = 18$, 5·5%), and two bla_{NDM} variants, $bla_{\text{NDM-1}}$ ($n = 10$, 3·1%) and $bla_{\text{NDM-23}}$ ($n = 8$, 2·5%). Carbapenemase genes were mostly detected in combination with ESBLs ($n = 276$, 84·7%), except for $bla_{\text{KPC-3}}$ genes ($n = 17$, 94·4%) and certain cases of $bla_{\text{OXA-48}}$ ($n = 27$, 10·6%) and $bla_{\text{VIM-1}}$ ($n = 6$, 16%). Whereas $bla_{\text{OXA-48}}$ was found in 21 different genetic backgrounds across hospitals, the other carbapenemase genes exhibited a strong association with specific hospitals and STs. For instance, KPC-3 was linked to ST512 and H5 and VIM-1 to ST147 and H7 (Fig. 3A).

To evaluate the genetic diversity within and divergence between 3GC- and carbapenem-resistant populations across hospitals, we estimated nucleotide diversity parameters (Supplementary Table 4, Additional File 4). We found a high diversity within the 3GC-resistant population across hospitals with an average nucleotide diversity (Dx) of 18,270 SNPs (range 14,899 −22,216, $\pi \sim 0·004$). These values were similar (t-test, p-value = 0·06) to the overall bacterial population (average Dx = 20,256 SNPs, range 18,124 −22,740, $\pi \sim 0.003$). Contrarily, the carbapenem-resistant population had a lower average nucleotide diversity of 13,224 SNPs (range 3,698 −19,997 SNPs, $\pi \simeq 0·003$) in comparison with both, the total and the 3GC-resistant populations (t-test, p-value = 0·004 and 0·02, respectively). Interestingly, the diversity of the carbapenem-resistant populations considerably

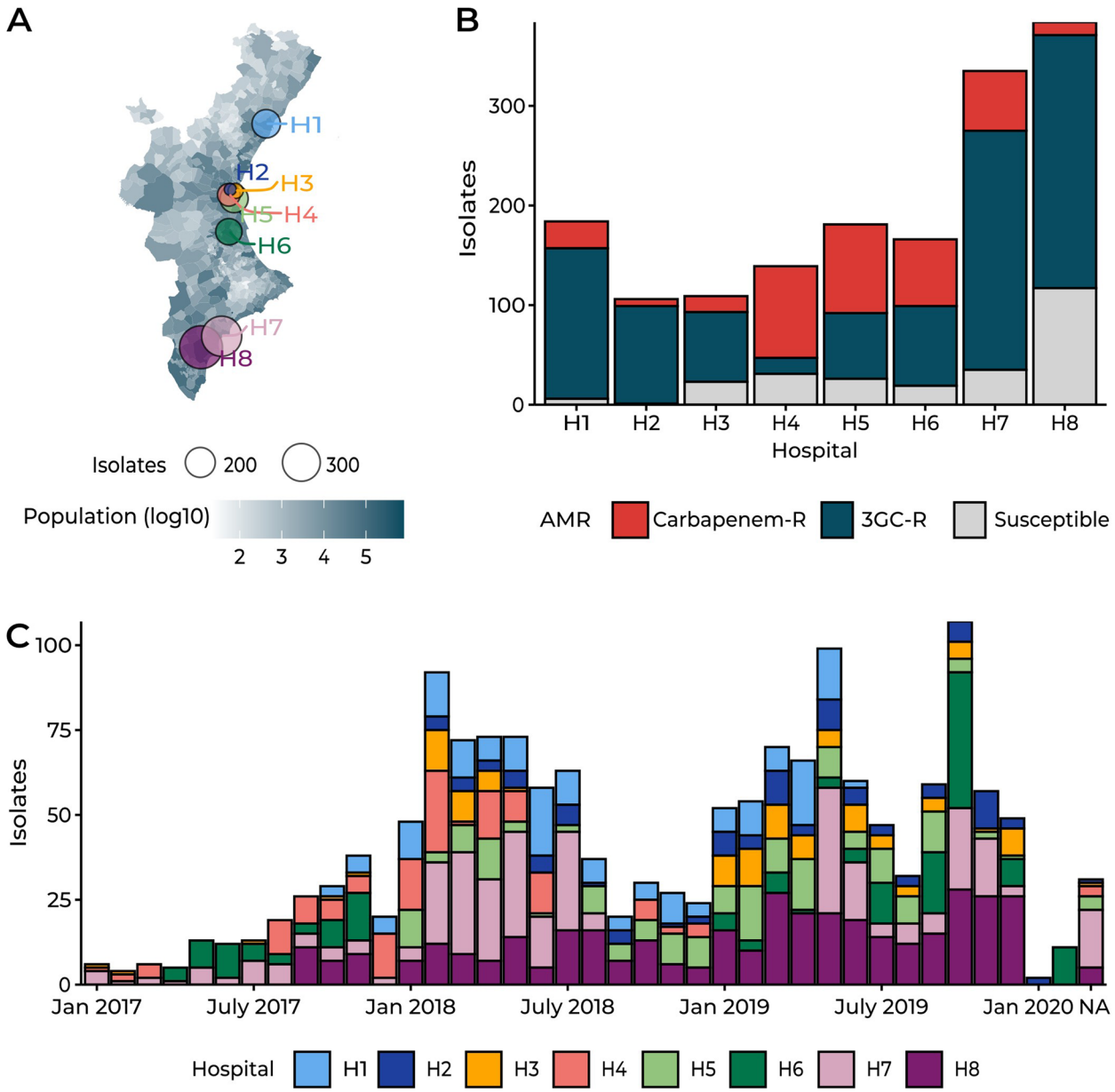
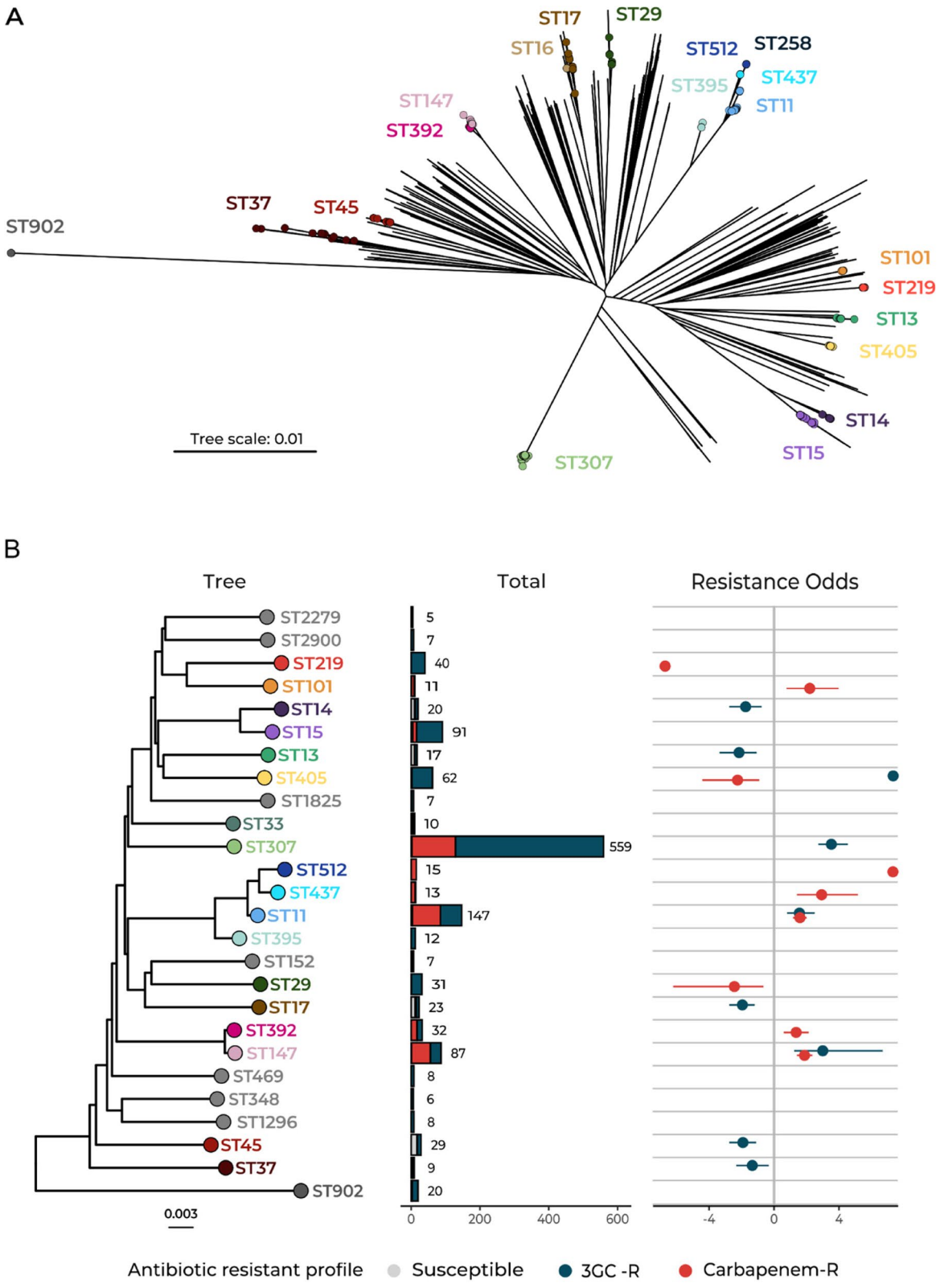


Fig. 1 **A** Surveillance of ***Klebsiella pneumoniae*** in the Comunitat Valenciana (SKPCV). Map illustrating the geographical distribution of hospitals participating in the SKPCV project and the total number of samples collected from each hospital. The map is color-coded based on population density. **B** Antimicrobial resistance (AMR) profiles of isolates from each hospital, categorized by resistance to third-generation cephalosporins (3GC-R), carbapenems (Carbapenem-R), or susceptibility to both. **C** Temporal distribution of SKPCV sample collection. NA: non- available dates

(See figure on next page.)

Fig. 2 **A** The SKPCV phylogeny. Maximum likelihood tree of the 1,604 genomes of the SKPCV project using the core-genome (90%) alignment (Supplementary Fig. 2). **B** Association between lineages and resistant types. A simplified version of the tree in (A) showing only those sequence types (STs) with at least five isolates. The bar plot depicts the total number of isolates within each sublineage, color-coded according to their antibiotic resistance profile: susceptible, resistant to third-generation cephalosporins, or carbapenems. The last column represents the odds ratio (OR) and its 95 percent confidence interval estimates for the association between each sublineage and resistance to either 3GC or carbapenems. Only statistically significant values (p-value < 0.005) determined by t-tests are shown. When confidence intervals (CI) could not be computed due to small sample sizes, the odds ratio (OR) is shown without CI



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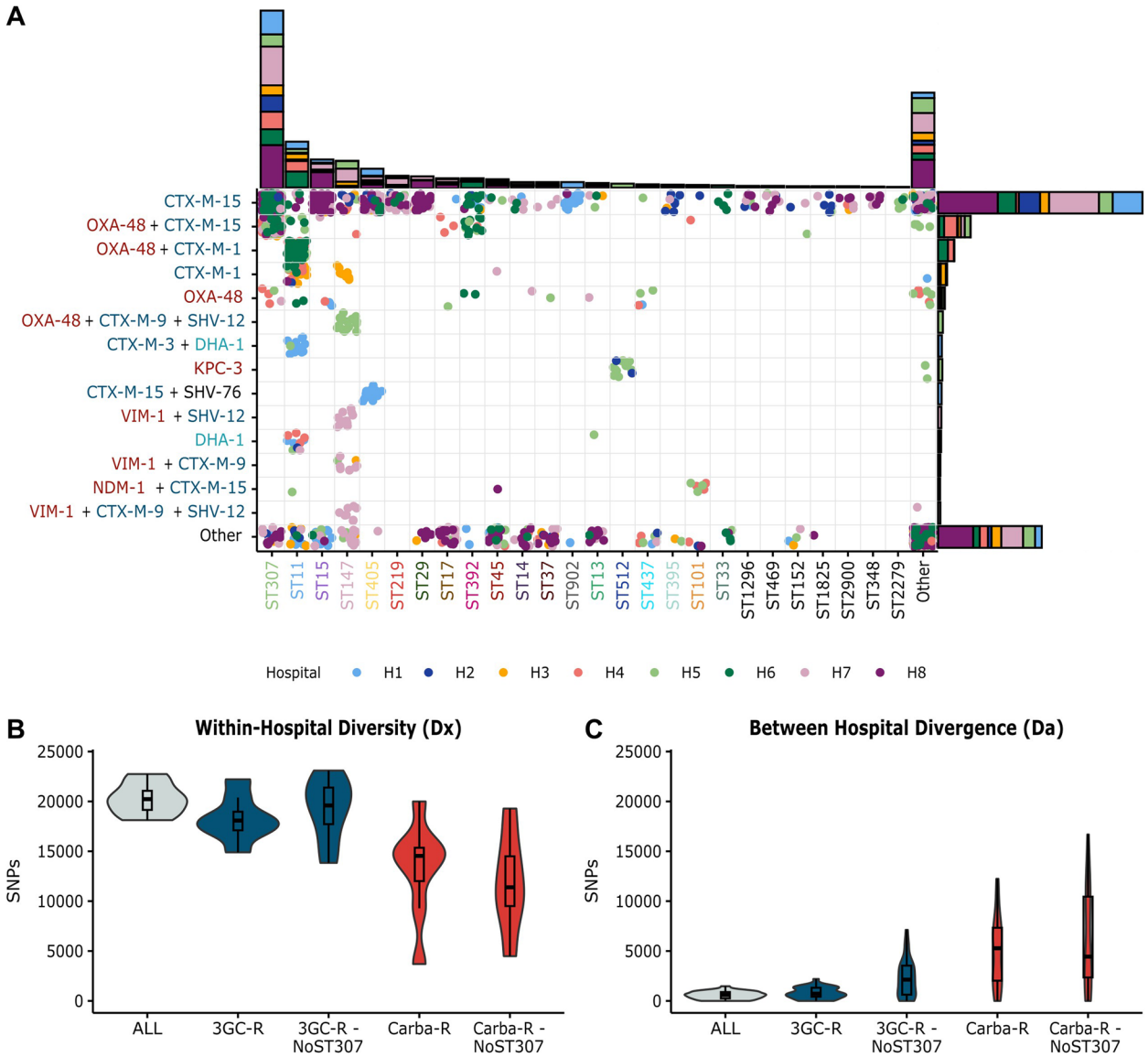

Fig. 3 **A** Co-occurrence of STs and AMR genes across different hospitals**.** Each dot represents an SKPCV isolate, colored according to its source hospital. The gene labels in the y-axis are color-coded to differentiate genes encoding extended-spectrum beta-lactamases (ESBLs) in dark blue, AmpC beta-lactamases (AMPCs) in light blue, and carbapenemases in red. **B** Genetic diversity within hospitals. The y-axis depicts nucleotide diversity measured by single nucleotide polymorphisms (SNPs) within each hospital for various sets: All isolates, the 3GC-resistant (3GC-R) population, 3GC-R population excluding ST307 isolates carrying $bla_{\text{CTX-M-15}}$ gene (3GC-R-NoST307), the carbapenem-resistant (Carba-R) population, and Carba-R population excluding ST307 isolates carrying $bla_{\text{CTX-M-15}}$ gene (Carba-R-NoST307). **C** Net between-hospital divergences. The y-axis represents net genetic divergence (Da) measured by SNPs for the same populations as in B

varied between hospitals with some exhibiting much more genetic diversity than others.

Regarding divergence between hospitals, the 3GC-resistant populations demonstrated high genetic similarity across hospitals, with an average divergence (Da) of 907 SNPs (range 67 – 2,196). In contrast, the carbapenem-resistant population showed higher genetic divergence in comparison (t-test, p-value < 0·001), with an average Da of 5,213 SNPs (range 0 – 12,235), reflecting the presence of distinct carbapenem-resistant lineages in different hospitals (Supplementary Tables 5–6, Additional File 4).

We repeated the analysis excluding the widespread and dominant ST307 lineage carrying the $bla_{\text{CTX-M-15}}$ gene (Supplementary Table 7, Additional File 4). The 3GC-resistant population showed similar genetic

diversity values within hospitals (average Dx = 20,271 SNPs, range 13,838 – 27,433; t-test, p-value = 0·255), but a significant greater divergence between hospital populations (average Da = 3,052 SNPs, range 281 – 18,141; t-test, p-value < 0·0001) (Fig. 3 B-C). This suggests that while the remaining 3GC-resistant population is highly diverse, certain lineages are confined to specific hospitals. We did not find differences for the carbapenem-resistant population (t-test, p-value = 0·08). This was expected as the ST307 population with resistance to carbapenems was exclusively associated to the $bla_{\text{OXA-48}}$ gene, thereby contributing minimally to genetic divergence.

Quantifying transmission

To characterize transmission groups (TGs) in the main lineages (n ≥ 5 isolates), we used high-resolution SNP comparisons (Supplementary Methods, Additional File 1).

Our analysis identified 173 TGs comprising 815 (50·8%) SKPCV isolates (Fig. 4A), of which 800 (98·1%) were resistant to 3GC, 249 (30·5%) were resistant to 3GC and

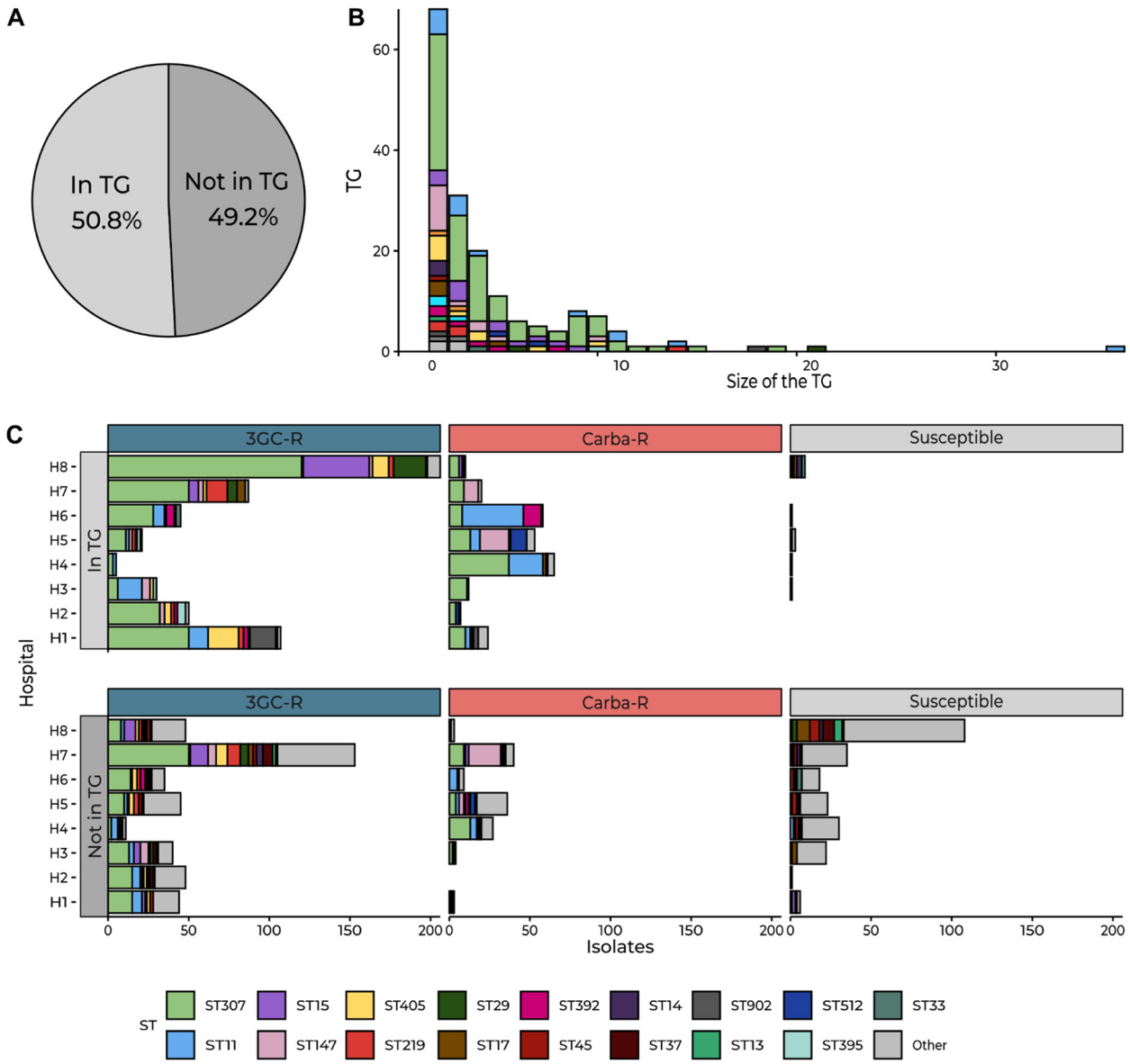

Fig. 4 Isolates in Transmission Groups. **A** Isolates included or not in Transmission Groups (TGs). **B** TG sizes. Distribution of the TGs size and the isolates involved. Groups are color-coded by Sequence Type (ST). **C** ST involved in TG and resistance in each hospital. Isolates categorized into TGs or not, and antimicrobial resistance patterns, within the different hospitals color-coded by ST

carbapenems, and 15 (1·8%) were sensitive. We found 20 large TGs, with 10 or more isolates (Fig. 4B). Some STs, mainly high-risk clones, were significantly associated with transmission, such as ST307, ST11, ST395, ST512, ST15, ST905, and ST29 (Fisher test, *p*-value < 0·05) (Fig. 4C). Remarkably, for ST11 and ST147, we found different TGs overlapping in time and hospitals (Supplementary Fig. 3 −5, Additional File 3).

We identified 122 TGs involving 460 SKPCV isolates restricted to a single hospital (intra-hospital). Among these, 8 were susceptible, while 308 and 144 were resistant to 3GC and carbapenems, respectively. Additionally, we identified 51 inter-hospital TGs (with isolates from more than one hospital) involving 355 SKPCV isolates, with 7 being susceptible, and 243 and 105 exhibiting resistance to 3GC and carbapenems, respectively (Fig. 5A). While ST307 and ST11 were major drivers of transmission across hospitals, other STs as ST512, ST905 were hospital-specific (Fig. 3C).

Most hospitals, except for a single pair, shared at least one TG (Fig. 5C, Supplementary Table 8, Additional File 4). However, the movement of isolates within TGs between hospitals was limited. Most TGs involved in inter-hospitals spread were mostly restricted to a main hospital, with an average of 1·76 cases (range 1 − 8 isolates) in other hospitals (Fig. 5D). Almost half of the inter-hospital TGs belonged to ST307 (n = 25, 49·01%), with a smaller representation of other 16 STs such as ST11 (n = 5), ST15 (n = 3), and ST395 (n = 3).

To further understand transmission dynamics of the 3GC-resistant and carbapenem-resistant populations, we analyzed the pairwise SNP distances of isolates from intra-hospital and inter-hospital TGs. Among the latter, we considered isolates collected in the same hospital (within-inter-hospital) or in different hospitals (between-inter-hospital) (Fig. 5B).

Notably, 3GC-resistant isolates showed a significant increase in average SNP distances when transmitted between hospitals than when transmitted within hospitals. Isolates transmitting within hospitals (intra-hospital TGs and within-inter-hospital TGs) displayed similar average SNP distances (9·3 vs 7·98 SNPs, p-value = 0·289). However, isolates from the same TG but from different hospitals (between-inter-hospitals) exhibited a significantly higher average SNP distance (12·4 SNPs) compared to both intra-hospital and within-inter-hospital TGs (p-values = 0·0004 and 0·0002, respectively) (Fig. 5B).

In contrast, isolates from carbapenem-resistant TGs showed no significant differences in average SNP distances across all categories (intra-hospital vs. within-inter-hospital vs. between-inter-hospital). The average SNP distances were 9·84 SNPs for intra-hospital TGs, 8·98 SNPs for within-inter-hospital TGs (p-value = 0·568 compared to intra-hospital), and 10·12 SNPs for between-inter-hospital TGs (p-values = 0·32 and 0·18 compared to intra-hospital and within-inter-hospital, respectively) (Fig. 5B).

Additionally, we included 11,529 local, national, and global genomes to evaluate the contribution to the ongoing regional transmission. We found 135 local and 67 global database genomes involved in the same TGs as the SKPCV genomes. Most were collected in the Comunitat Valenciana (n = 47) (not in the SKPCV project) or other parts of Spain (n = 150) from 2014 to 2018, suggesting that there was a previous national and regional spread of some of the lineages found in our study. However, as we increased the SNP threshold for defining TGs (Fig. 4E), some global isolates were gradually included in them, particularly from European countries such as Greece, United Kingdom, France, Italy, and Portugal.

The successful ST307 lineage

ST307 was the most frequent lineage in the SKPCV collection (n = 559, 34·8%), mainly related to the spread of $bla_{\text{CTX-M-15}}$ as shown above, and a major driver of within and between hospitals transmission, of both 3GC- (n = 405, 49·0%; Fisher test, p-value < 0·0001) and carbapenem resistance (n = 127, 34·2%) (Supplementary Fig. 6, Additional File 3).

We obtained a global phylogeny of the lineage (Fig. 6) including all ST307 isolates collected in this work and in the local and global databases (n = 1,110 genomes). Most of these were collected in Europe (n = 173) or Spain (n = 113), while a smaller number were sampled from other locations (n = 111) or lacked geographic data (n = 154). We found that the ST307 is highly conserved regardless of the source, geographic origin, or subclade (Fig. 5). It showed an average pairwise distance of 103·2 SNPs (range 0 − 1,123). Notably, 978 (88·1%) of the global ST307 genomes carried the $bla_{\text{CTX-M-15}}$ ESBL gene. The $bla_{\text{OXA-48}}$ carbapenemase gene was found in a few subclades in the ST307 tree, mostly in SKPCV clades.

To understand the genomic environment of the $bla_{\text{CTX-M-15}}$ and the $bla_{\text{OXA-48}}$ genes in this lineage, we sequenced some SKPCV isolates using long reads. We found the $bla_{\text{CTX-M-15}}$ in a well-described MDR IncFIBk/IncFIIk plasmid [20]. This plasmid is conjugative with a multidrug resistance region carrying *strA*, *strB, aac(3)-IIa, aac(6')-Ib-cr, qnrB1 sul2, dfrA14, tet(A),* $bla_{\text{TEM-1B}}$, $bla_{\text{CTX-M-15}}$, and $bla_{\text{OXA-1}}$ genes (Supplementary Fig. 7, Additional File 3). It also carries copper- and arsenic-resistance genes. This plasmid was found with an average coverage of 73·1% in the $bla_{\text{CTX-M-15}}$ carriers (range 1 − 100). The $bla_{\text{OXA-48}}$ carbapenemase gene was found in the also well-described IncL pOXA-48a plasmid [21]

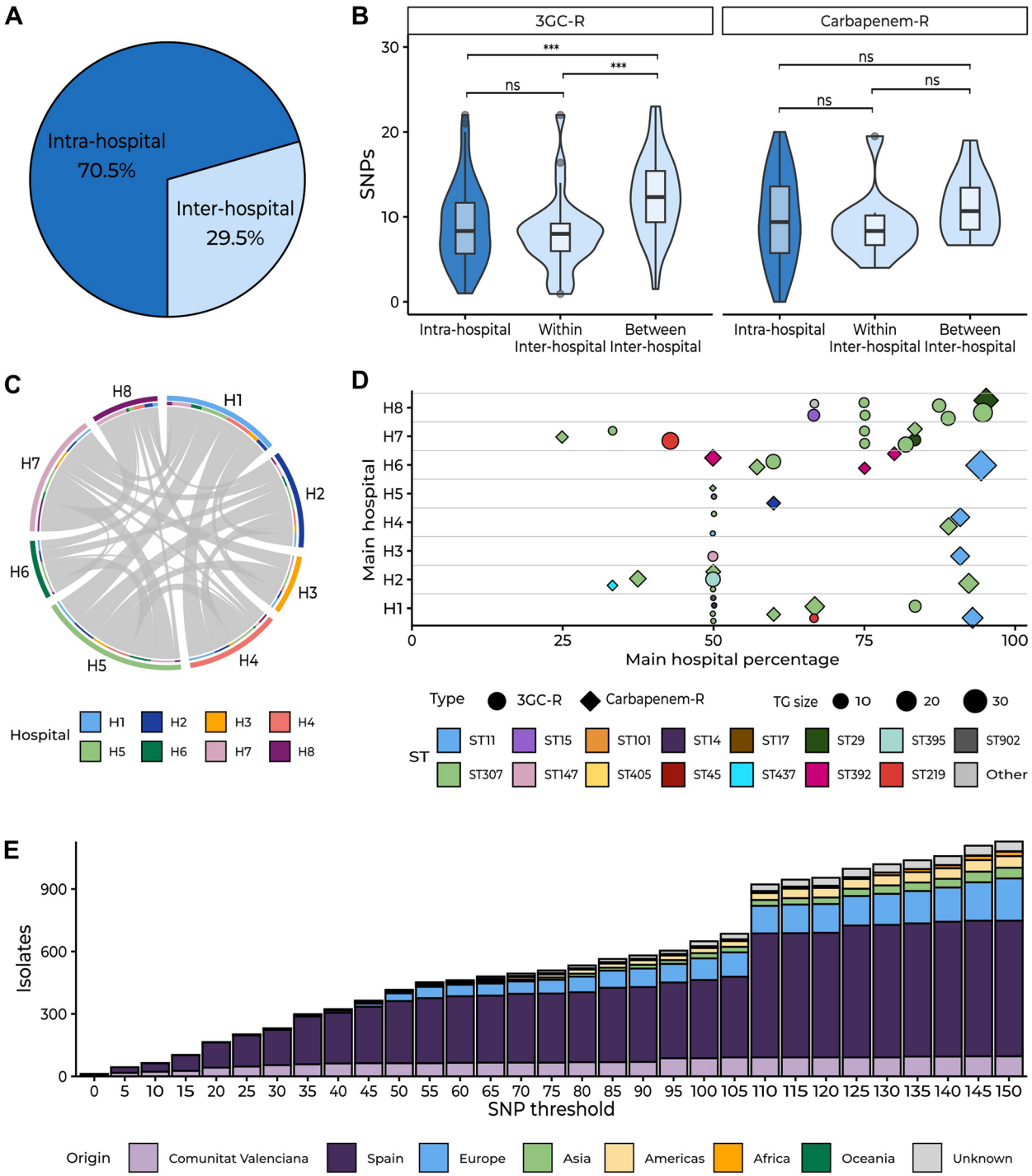

Fig. 5 **A** Proportion of Transmission Groups (TGs) in intra-hospital or inter-hospital transmission. **B** Single Nucleotide Polymorphisms (SNPs) between TG in intra-hospital or inter-hospital transmission. Distribution of pairwise SNPs between SKPCV isolates in TGs limited to a single hospital (intra-hospital) or involving inter-hospital transmission, dividing the latter category into isolates collected in the same hospital (within-inter-hospital) and in different hospitals (between-inter-hospital). **C** TGs shared by hospitals. **D** Percentage of isolates within inter-hospital TG shared between hospitals. Each point represents a TG, with the y-axis representing the main hospital and the x-axis indicating the percentage of samples in the TG attributed to that hospital. The size of the dot reflects the size of the TG, the shape of the dots represents the resistant type, and dots are color-coded by Sequence Type (ST). **E** Global isolates included in SKPCV TGs. Local (Comunitat Valenciana), national (Spain), and global genomes included in SKPCV TGs as the SNP threshold for defining TGs increases

carrying no other resistance genes with an average coverage of 91·8% (range 30 – 95%).

SKPCV isolates were not grouped in a single clade; instead, they were found dispersed in different clades which were mainly formed by SKPCV and a few European isolates. Moreover, these clades corresponded to the groupings of different TGs identified in the different hospitals (Fig. 6, Supplementary Fig. 8, Additional File 3). This suggests that the current distribution of ST307 in the CV is due to multiple introductions of the lineage into the CV hospitals followed by subsequent intra- and inter-hospital transmissions.

Discussion

In this study, we investigated the regional spread of 3GC- and carbapenem-resistant

K. pneumoniae by sequencing and analyzing 1,604 isolates from 2017 to early 2020. These isolates were collected from eight reference hospitals in the CV region, representing, to our knowledge, the largest dataset analyzed at a regional scale [10, 12, 22–25]. We employed high-resolution SNP comparisons to assess genomic diversity and transmission dynamics, enhancing our understanding of the transmission patterns driving the AMR spread in the region.

Our data revealed two different scenarios for the emergence and spread of 3GC- and carbapenem-resistant strains in the region. The 3GC-resistance population was very diverse with high prevalent lineages spreading across hospitals (e.g., ST307 carrying the $bla_{\text{CTX-M-15}}$ ESBL) and low prevalence lineages restricted to single hospitals (e.g., ST11 carrying $bla_{\text{DHA-1}}$ and $bla_{\text{CTX-M-1}}$). In contrast, carbapenem resistance was associated with distinct carbapenemase genes and genetic backgrounds in each hospital, with low transmission between them.

Notably, intra-hospital transmission was the primary driver of dissemination for both resistant types, yet we found distinct patterns of inter-hospital transmission for each. 3GC-resistant strains showed higher diversity in inter-hospital than in intra-hospital transmission, a pattern not observed for carbapenem resistance. These findings suggest that the inter-hospital transmission of 3GC-resistance between hospitals likely involves intermediate steps, such as undetected carriers within the community [5, 6], while carbapenem resistance transmission appears to be more direct, probably by patient sharing, a known contributor to regional AMR dissemination [13].

These findings are in line with a broader, global trend, where 3GC-resistance is already prevalent in healthcare settings and the community [3], contrasting with the relatively recent emergence of carbapenem resistance in hospitals, which is now spreading rapidly [11, 22]. The latter poses a significant concern both globally and in the studied region as we have observed sporadic dissemination of carbapenem-resistant strains to other facilities, which has been shown to serve as precursor for a wider spread [13]. Indeed, an epidemiological study conducted in this region [25] has shown a concerning trend of *K. pneumoniae* carbapenem-resistant infections shifting from tertiary hospitals to smaller healthcare settings, including primary care.

Interestingly, both 3GC- and carbapenem resistance coexisted in certain lineages, suggesting that carbapenem resistance may emerge in genetic backgrounds already exhibiting 3GC-resistance. In our dataset, this is evident for the carbapenem $bla_{\text{OXA-48}}$ gene, which was detected in previously reported 3GC-resistant lineages such as ST307 and ST11. However, other carbapenemase genes were introduced in the CV with their carrier lineages. Examples include $bla_{\text{KPC-3}}$ with ST512, bla_{NDM} with ST437, or bla_{VIM} with ST147. The different emergence patterns between OXA-48 and other carbapenemases may be likely influenced by the prevalence of these genes and lineages in the region and the transfer characteristics of the plasmids carrying these genes. The $bla_{\text{OXA-48}}$ gene is located in a highly transferable plasmid [21], which, combined with its abundance and the high prevalence of ST307 and ST11 lineages in the region [22, 26], creates favorable conditions for plasmid dissemination [27]. In contrast, carbapenemases such as KPC, VIM, and NDM show lower prevalence in the region [22], and primarily disseminated through clonal expansion [28–30].

Although most of the identified STs belong to global high-risk clones [3], the contribution of global isolates to the spread of AMR in the region was limited. Although this could be due to the intrinsic bias of the database genome collection, it is worth noting that we have included over 11,000 genomes in our analysis from at least 93 countries. This highlights that efforts

(See figure on next page.)

Fig. 6 Global phylogeny of lineage ST307. Maximum likelihood phylogenetic tree of ST307 genomes (n = 1,110). The core genome at 90% had 4,393 genes and resulted in an alignment of 4,268,326 bp with 16,936 variant positions (~ 3,967 SNPs/Mbp). Clades containing SKPCV isolates are highlighted in gray. Dots at the end of the tips are colored by the hospital where they were collected. The inner ring depicts the geographic origin. The second ring marks the presence or absence of the $bla_{\text{CTX-M-15}}$ and the third the coverage of the pCTX-M-15 assembled in this work. The fourth is the presence of $bla_{\text{OXA-48}}$ whereas the outermost ring is the coverage of the pOXA-48 assembled in this work

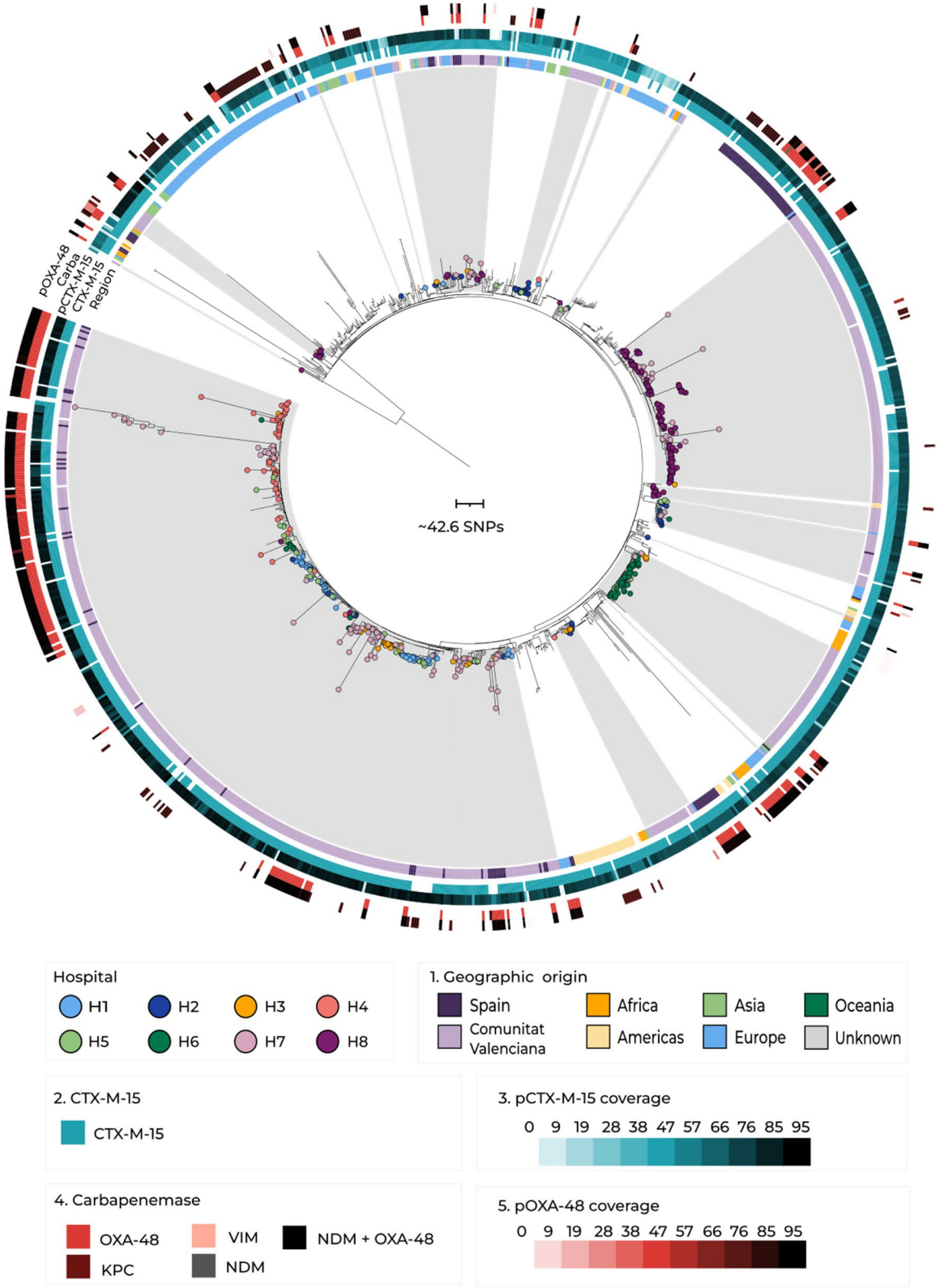

Fig. 6 (See legend on previous page.)

to share genomic data should be intensified to facilitate the exploration of potential transmission routes between countries.

We identified lineage ST307 as a major driver of 3GC- and carbapenem-resistant infections and transmissions in the region. This lineage is a recognized high-risk clone strongly associated with the $bla_{\text{CTX-M-15}}$ ESBL gene, which is increasing its prevalence worldwide [3, 20]. Our global phylogenetic analysis revealed that ST307 is highly conserved with multiple distinct subclades linked to diverse geographic origins. We observed cases of ST307 of the CV region in distinct clades, suggesting recurrent introductions in the region. The regional and global success of this clone despite its low genetic diversity suggests a recent global expansion of the lineage, underscoring the importance of monitoring its spread. However, further analyses are needed to fully understand the global evolution of this clade.

While this study provides valuable insights into nosocomial *K. pneumoniae* transmission, we lacked information of community cases which hinder potential transmission routes between hospitals and the community. This prevents us from fully understanding the factors underlying the observed differences in the spread of 3GC-resistant and carbapenem-resistant strains, highlighting the need of incorporating community surveillance in future studies.

Conclusions

Our investigation has revealed different transmission dynamics in 3GC- and carbapenem-resistant strains of *K. pneumoniae*, emphasizing the pivotal role of intra-hospital dissemination, and highlighting the significant contribution of ST307 carrying the $bla_{\text{CTX-M-15}}$ gene to the escalation of 3GC- and carbapenem resistance in the CV region and globally. These findings are crucial for implementing tailored and effective measures for the control of antimicrobial resistant *K. pneumoniae* in the region. Further studies in other regions will be required to assess how these findings apply to the dynamics of AMR globally. Altogether, regional and global efforts are required to address the challenge of antimicrobial resistance.

Abbreviations

3GC	Third-generation Cephalosporins
AMR	AntiMicrobial Resistance
Bp	Base pairs
CG	Clonal Groups
cgMLST	Core genome MultiLocus Sequence Typing
CV	Comunitat Valenciana
Da	Nucleotide divergence
Dx	Nucleotide diversity
ESBL	Extended-Spectrum β-lactamase
Kbp	Kilo base pairs
SKPCV	Surveillance of Klebsiella pneumoniae in the Comunitat Valenciana
SL	SubLineages
SNP	Single Nucleotide Polymorphism
SRA	Sequence Reads Archive
ST	Sequence Type
TG	Transmission Group
π	Nucleotide diversity per site

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13756-025-01553-2.

Additional file 1: Supplementary Methods

Additional file 2: Supplementary Results

Additional file 3: Supplementary Figures

Additional file 4: Supplementary Tables

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The Networked Laboratory for the Surveillance of Antimicrobial Resistance of the Valencian Community:
Neris García-González and Fernando González-Candelas. Joint Research Unit Infection and Public Health FISABIO-University of Valencia, Institute for Integrative Systems Biology (I2SysBio). CIBER in Epidemiology and Public Health. Valencia. Spain.
Begoña Fuster, Nuria Tormo, Carme Salvador, and Concepción Gimeno. Microbiology Service. Consorcio Hospital General Universitario. Valencia, Spain.
Victoria Domínguez. Microbiology Service. Hospital Arnau de Vilanova. Valencia, Spain.
Salvador Giner. Microbiology Service. Hospital Universitario y Politécnico La Fe. Valencia, Spain.
Javier Colomina and David Navarro. Microbiology Service. Hospital Clínico Universitario. Valencia, Spain.
Llúcia Martínez. Sequencing Platform. FISABIO. Valencia, Spain.
Antonio Burgos and Olalla Martínez. Microbiology Section. Hospital La Ribera, Alzira, Spain.
Bárbara Gomila-Sard and Rosario Moreno. Microbiology Service. Hospital General Universitario. Castelló, Spain.
Inmaculada Vidal, Antonia Sánchez and Juan Carlos Rodríguez. Microbiology Service. Hospital General Universitario. Alacant, Spain.

Authors' contributions

NGG collected, curated, interpreted, and visualized the data, conducted the formal analyses, wrote the original and final drafts, and conceptualized the study. JSF curated data. The NLSAR working group, VSZ, IV, JCR, BF, NT, CS, CG, BGS, SG, OM, JC, DN, and VD provided resources. BB contributed to visualization and reviewed the final draft. FGC conceptualized the study, obtained the funding and administered the project, supervised all the process, and reviewed and edited the final draft. All the authors contributed to the revision and editing of the paper, and all of them approved the final version and its publication.

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Data availability

The sequencing reads generated have been deposited at the European Nucleotide Database (ENA) under project number PRJEB37504 and the accessions listed in Supplementary Tables 1 and 2, Additional File 4. All the scripts necessary to perform the described analyses are available on GitHub at https://github.com/NerisGarcia/Klebsiella_transmission.

Declarations

Ethics approval and consent to participate

The results reported make no use of individual information and were obtained under a routine surveillance project which the DGSP-FISABIO ethics committee declared to be exempt from approval.

Competing interests

The authors declare no competing interests.

Author details

[1]Unidad Mixta Infección y Salud Pública FISABIO-Univ. València, Instituto Biología Integrativa de Sistemas (CSIC-UV), Valencia, Spain. [2]Synthetic Biology Unit, Institut Pasteur, Paris, France. [3]Hospital General Universitario de Elche, Elche, Spain. [4]Hospital General Universitario Dr. Balmis, Alicante, Spain. [5]Consorcio Hospital General Universitario de Valencia, Valencia, Spain. [6]Dpto. Microbiología y Ecología, Universitat de València, Valencia, Spain. [7]Hospital General Universitario de Castelló, Castelló, Spain. [8]Hospital Universitario y Politécnico La Fe, Valencia, Spain. [9]Hospital de La Ribera, Alzira, Spain. [10]Hospital Clínico Universitario de Valencia, Valencia, Spain. [11]CIBER en Enfermedades Infecciosas, Madrid, Spain. [12]Hospital Arnau de Vilanova, Valencia, Spain. [13]CIBER en Epidemiología y Salud Pública, Madrid, Spain.

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