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Population structure of OXA-48-producing *Klebsiella pneumoniae* ST405 isolates during a hospital outbreak characterised by genomic typing

Elena López-Camacho[a], José Ramón Paño-Pardo[b,c,1], Guillermo Ruiz-Carrascoso[a], Jan-Jaap Wesselink[d,2], Silvia Lusa-Bernal[d], Ricardo Ramos-Ruiz[e], Susana Ovalle[e], Rosa Gómez-Gil[a], Verónica Pérez-Blanco[f], María Pérez-Vázquez[c,g], Paulino Gómez-Puertas[h], Jesús Mingorance[a,c,*]

[a] *Servicio de Microbiología, Hospital Universitario La Paz, IdiPAZ, Paseo de la Castellana 261, 28046 Madrid, Spain*
[b] *Unidad de Microbiología Clínica y Enfermedades Infecciosas, Hospital Universitario La Paz, IdiPAZ, Paseo de la Castellana 261, 28046 Madrid, Spain*
[c] *Red Española de Investigación en Patología Infecciosa (REIPI), Spain*
[d] *Biomol-Informatics, S.L., Campus Universidad Autónoma de Madrid, C/Faraday 7, 28049 Madrid, Spain*
[e] *Madrid Science Park, Campus Universidad Autónoma de Madrid, C/Faraday 7, 28049 Madrid, Spain*
[f] *Servicio de Medicina Preventiva, Hospital Universitario La Paz, IdiPAZ, Paseo de la Castellana 261, 28046 Madrid, Spain*
[g] *Centro Nacional de Microbiología, Instituto de Salud Carlos III, Majadahonda, 28220 Madrid, Spain*
[h] *Centro de Biología Molecular 'Severo Ochoa' (CSIC-UAM), Campus Universidad Autónoma de Madrid C/Nicolás Cabrera 1, 28049 Madrid, Spain*

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ABSTRACT

Objectives: The aim of this study was to investigate the structure of a broad and sustained hospital outbreak of OXA-48-producing *Klebsiella pneumoniae* (KpO48) belonging to sequence type 405 (ST405).
Methods: Whole-genome sequencing and comparison of ten ST405 KpO48 isolates obtained from clinical samples in our hospital was performed. Using stringent criteria, 36 single nucleotide polymorphisms (SNPs) were detected (range 0–21 in pairwise comparisons), and allele-specific PCR was used to call the SNPs among a larger set of isolates.
Results: Several haplotypes were identified within the population. The haplotypes did not show a spatial structure, but a temporal evolution of sequential haplotype replacements was observed.
Conclusions: The dispersed spatial distribution suggests a reservoir formed by a large pool of colonised patients, and the temporal replacement pattern suggests that the sustained outbreak was composed of several small outbreaks that appeared and rapidly dispersed to several units.

1. Introduction

OXA-48 and related enzymes are class D β-lactamases that hydrolyse most β-lactam antibiotics, including carbapenems. They are produced by different enterobacterial species and generally appear combined with other antimicrobial resistance mechanisms [1,2]. In most cases the bla_{OXA-48} gene is found in a composite transposon (Tn*1999*) in a single 62-kb IncL conjugative plasmid that transfers very efficiently within and between species [3,4]. This plasmid has spread during the last years throughout Europe [5–8], most often associated with OXA-48-producing *Klebsiella pneumoniae* (KpO48). In our hospital (Hospital Universitario La Paz, Madrid, Spain), KpO48 were first detected in 2010 [9], simultaneously with its emergence in several other hospitals in Spain [10]. During the first 2 years of the outbreak, most OXA-48-producing isolates were *K. pneumoniae* belonging to multilocus sequence typing (MLST) sequence type 405 (ST405), with a few sporadic *K. pneumoniae* isolates belonging to other MLST types and a few other enterobacterial species. Later on ST11 became the major group [11]. This epidemic has been characterised in our hospital by a sustained and complex pattern of OXA-48-producing isolates

* Corresponding author at: Servicio de Microbiología, Hospital Universitario La Paz, IdiPAZ, Paseo de la Castellana 261, 28046 Madrid, Spain.
E-mail address: jesus.mingorance@idipaz.es (J. Mingorance).
[1] Present address: Servicio de Enfermedades Infecciosas, Hospital Clínico Universitario 'Lozano Blesa', Instituto de Investigaciones Sanitarias Aragón, Avenida San Juan Bosco 15, 50009 Zaragoza, Spain.
[2] Present address: Diagenode S.A., Liège Science Park, Rue Bois Saint-Jean 3, B-4102 Seraing, Belgium.

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belonging to two major and several minor STs that appear scattered throughout the hospital [9,11,12]. The repeated isolation of the same ST in some hospital units may suggest that local (i.e. within-unit) transmission might play a dominant role in maintaining an endemic situation in these units. To test this hypothesis, a high-resolution single nucleotide polymorphism (SNP) analysis of ST405 KpO48 isolates recovered from clinical samples during the first 2 years of the outbreak was performed.

2. Materials and methods

2.1. Setting and strains

Hospital Universitario La Paz is a third-level academic centre that provides medical assistance to a mixed urban and rural population of ca. 600 000 people in the north area of Madrid. This study included all KpO48 isolates belonging to ST405 and obtained from clinical samples between December 2010 and December 2012. One isolate per patient was included. Data on isolation unit, date and sample type were collected. No other patient data were registered. One isolate obtained in April 2010 was identified retrospectively and was recovered from the collection of the Microbiology Service. Clonality was established using DiversiLab® [9] and by ST405-specific PCR typing [13].

2.2. Genome sequencing

The genome of isolate *K. pneumoniae* KpO3210 (GenBank accession no. **AMRH00000000**) was used as the reference to call for SNPs [14]. To improve the assembly, the genome was resequenced from a single-read shotgun library and two long-insert paired-end libraries (3 kb and 8 kb) using GS Junior Titanium Chemistry and a GS Junior Sequencer (Roche Applied Science, Penzberg, Germany). These sequencing runs were assembled using Newbler 3.0 into five scaffolds (30 contigs), with an estimated genome size of 6.3 Mb and an N_{50} of 510 549 bp (GenBank accession no. **AMRH02000000**). To study the genetic diversity of the isolates at the subclonal level, nine ST405 KpO48 isolates from different wards and spanning the period from December 2010 to December 2012 were selected. The nine isolates were shotgun-sequenced using the same methodology in an Illumina GAIIx sequencer (one of them in 2 × 125 and eight in 1 × 75). Paired-end sequencing of strain Kp2 produced 2 × 18 464 504 reads; the single read sequencing produced an average of 5 485 377 reads per genome. SNP calling and a core-SNP alignment were done using either paired-end or single-end reads with Snippy v.3.0 [15]. SNPs were selected using stringent criteria: detection in 100% of the reads in both directions and minimum depth equal to or higher than the mean coverage. All SNPs were confirmed by Sanger sequencing. The SNPs were called using Illumina assembly (accession no. **AMRH00000000**) [14] but have been renumbered according to **AMRH02000000**. A minimum spanning tree of SNP profiles was constructed with Sneato v.2 (http://user.xmission.com/~wooding/Sneato/).

2.3. Allele-specific endpoint PCR

To determine the SNP variants in non-sequenced isolates, an allele-specific endpoint PCR strategy was used. For every SNP, three PCR primers were designed: one primer located ca. 100–200 bp away from the polymorphic position, and two SNP primers with the 3′ end in the variant position (one primer with each possible variant sequence) (Supplementary Table S1). PCR reactions were designed to allow amplification only when there was a perfect match, so that each isolate would yield a PCR product only with one of the two SNP primers. PCR products were detected by agarose gel electrophoresis (Supplementary Fig. S1). The primers were tested with the sequenced strains. SNPs were recorded in Microsoft Excel (Microsoft Corp., Redmond, WA), were concatenated and were transformed to FASTA format and analysed using MEGA7 [16].

3. Results

The genome of KpO48 isolate KpO3210 was resequenced using the Junior 454 system with one single-read and two paired-end libraries of 3 kb and 8 kb length. The revised assembly of the genome of KpO3210 contains five scaffolds: the chromosome and four plasmids. All four plasmid scaffolds could be mapped with high coverage (>96%) and identity (>98%) to plasmids present in the GenBank database (Table 1). Scaffold 2 contains a multidrug resistance region that includes the $bla_{\text{TEM-1}}$, $bla_{\text{CTX-M-15}}$ and $bla_{\text{OXA-1}}$ β-lactamase genes, the *aac(6′)-Ib*-cr and *aacC3* aminoglycoside resistance genes, and a *qnrB1* gene, among others.

The genomes of nine additional ST405 KpO48 isolates obtained from different wards and selected to span the period from December 2010 to December 2012 were sequenced and the reads were mapped to the KpO3210 genome. Isolate Kp2 had lost the $bla_{\text{OXA-1}}$ and *aac(6′)-Ib*-cr genes, and Kp5 had lost the *qnrB1* gene, although they still had the rest of scaffold 2. Isolates Kp3 and Kp6 had lost scaffold 4, whilst isolate Kp9 had an additional scaffold, highly similar to the *Klebsiella oxytoca* plasmid pKO_JKo3_2 [18] (Table 1).

Using stringent criteria, 36 SNPs were identified and were confirmed by Sanger sequencing (Table 2), of which 5 were in intergenic regions and 31 were in coding regions (12 in the first codon position, 15 in the second codon position and 4 in the third codon position). Of the 31 SNPs in coding regions, 26 were non-synonymous and 5 were synonymous. The variants found in coding regions were localised in genes coding for a variety of products including enzymes, regulatory proteins, transporters and hypothetical proteins. Alignment of the concatenated SNPs showed 16 non-phylogenetically informative variants (those that appear just once in one of the sequenced strains [16]) and 20 phylogenetically informative ones (Table 2). Pairwise comparisons showed between

Table 1
Plasmids identified in *Klebsiella pneumoniae* isolates Kp1–10.

	Size (kb)	Inc[a]	Copy number[b]										Most similar plasmid	GenBank acc. no.
			Kp1	Kp2	Kp3	Kp4	Kp5	Kp6	Kp7	Kp8	Kp9	Kp10		
Scaffold 2	233 020	FIB/FIIB	2	2	1	1	2	1	1	1	2	1	pKPN3-307_typeA	**KY271404.1**
Scaffold 3	63 400	L	1	2	1	2	1	1	2	2	1	1	E71T	**KC335143.1**
Scaffold 4	34 327		3	4	0	10	1	0	5	4	3	4	pECAZ146_3	**CP018988.1**
Scaffold 5	4593	Col440I	9	7	8	32	12	11	7	12	7	12	pEC08-5	**JX238444.1**
	104 331										1		pKO_JKo3_2	**AP014953**

[a] The Inc type is proposed on the basis of sequence similarity identified with PlasmidFinder [17].
[b] Numbers refer to copy numbers in the sequenced strains estimated from the ratio of the mean coverage for each scaffold to the mean coverage of scaffold 1.

Table 2
Single nucleotide polymorphisms (SNP) set used for typing of OXA-48-producing *Klebsiella pneumoniae* ST405 isolates. The table shows the SNP positions in the KpO3210 genome sequence (GenBank accession no. **AMRH02000000**), the polymorphic variants in parenthesis with their surrounding sequences, as well as the amino acid changes, locus tag and GenBank annotation.

SNP	Contig	Position	Sequence	locus_tag	AA change	Protein (GenBank)	Phylogenetically informative*
SNP1	sctg_0001_0005	1620893	GCCAAATTGTCGTAGTGAGC(T/A) CGGATCCGAGTAGTTAGGGT	C630_12920	–	Hypothetical protein	Y
SNP2	sctg_0001_0005	125482	TCGACTCCAGCCAGTTTAAT(G/T) GCTCCATTCCGCAAATTTTC	C630_05405	G238C	Pseudogene	Y
SNP3	sctg_0001_0011	385346	ACCCGTTATTAATGCGGGGG(T/A) AATGATGGCAAACATCATTT	C630_17345	T73S	Ammonium transporter	Y
SNP4	sctg_0001_0005	1613607	CAGCGAGATCGAGTAATCTG(A/T) GTGCTTTTACGCCTCCTGCG	C630_12895	L171H	Tyrosine permease	N
SNP5	sctg_0001_0005	1549284	CGCTCGCGCTACCAGGTGCA(T/G) ATAGAGTGCCTGAGTACGGA	C630_12670	H402Q	Hypothetical protein	Y
SNP6	sctg_0001_0002	35155	GGTCTGCCGCGGTTTGACAA(C/A) CAGCTCAACTCCGGTAAGGA	C630_01335	V32F		Y
SNP7	sctg_0001_0002	339756	CGCAATGGCCTGGGCGATGG(C/T) CAGGATATGCGGTTCGTTGA	C630_02910	A63T	Phosphoglucomutase	Y
SNP8	sctg_0001_0017	34653	GGTGGCCCGGCGGCCGCGTC(G/A) CCAGCCGCGAGGCATAGCGC	C630_22240	A131V	AAA family ATPase	N
SNP9	sctg_0001_0014	267244	TGGGGCTGCTGGGCGGCGCT(G/A) CGGCGATCTTTGACGTCTGG	C630_20845	A98T	Ribose ABC transport system, permease protein RbsC	N
SNP10	sctg_0001_0005	1050633	GATATTTAACGGGGCGCCGG(C/G) TGTTCCAGTGCGTACCGTCT	C630_10155	A85G	VgrG protein	Y
SNP11	sctg_0001_0005	658096	AGTCTTACCACGGGATGCTG(G/A) CCTGCGTCATCGCCGGCGCC	C630_08065	A223T	LysR family transcriptional regulator YneJ	Y
SNP12	sctg_0001_0004	57177	GAGCGAGGGTAAAGATACCT(T/A) TAGCGCCGGTTATCAGCAGG	C630_04620	F219Y	Glucose-1-phosphatase	N
SNP13	sctg_0001_0005	322733	GGGTGACGGTCAACTCCCTG(A/T) CCATTTGGCCAGCGTCGGGC	C630_06395	V689A	Glycoside hydrolase	N
SNP14	sctg_0001_0005	1556836	CTGGGGATTAACGTGAGTCG(T/C) CTGCGCATCGAGATTTTTCT	C630_12705	–	ABC transporter permease	Y
SNP15	sctg_0001_0009	157189	TACCAGCAGAGCCGGTACCA(G/A) GAGGGACAGTACTTTAACTT	C630_13915	–	OmpK36 porin	N
SNP16	sctg_0001_0005	1027559	CGTTGCCCCCGTTCTGACGG(A/T) TCCGTTCGCAGGCTTCGGCG	C630_10025	I89N	Lactoylglutathione lyase	Y
SNP17	sctg_0001_0002	188689	CGCGGAAGCAGGGGTTTTCG(A/T) CGCCAATGCGGGTGGTGGAA	C630_02185	V221D	GntR family transcriptional regulator	Y
SNP18	sctg_0001_0005	1476482	CAATACGCTGGCGGTAACCG(G/A) CGAAGCCTTCTCTCGTCAGG	C630_12340	G216D	Cystine transporter subunit	Y
SNP19	sctg_0001_0014	338443	CGTGACGCCCTGTAAAAATG(A/G) CACCGAACCGACGATCTGCC	C630_21195	S55P	Chloride channel protein	N
SNP20	sctg_0001_0017	28769	CCGCATACGCCGCAGCCGGT(G/A) CGCCCGGCCAGCGCGCGACG	C630_22200	–	Sulfurtransferase FdhD	N
SNP21	sctg_0001_0001	223506	CCGGCCCCGCCAGCAGCGCG(A/C) TCTCCCGATGCCCCATCTCA	C630_01125	I184S	*lacI*	N
SNP22	sctg_0001_0023	207222	CAATCTTGTTTTCCATCAAT(T/G) TTACGAAGAGATGCGCATCA	C630_24275/ C630_24280	–	Intergenic	Y
SNP23	sctg_0001_0002	255908	TGGCGGTAATGCTGGCCTTC(C/G) GCACGCCCTTTGTTGACCAC	C630_02480	–	4-Phytase/acid phosphatase	Y
SNP24	sctg_0001_0002	173769	TATCTTCACTCTTTGGACGA(G/T) CCACTACTTTTTTCCTCACG	C630_02125	A2D	Transcriptional regulator, TetR family	N
SNP25	sctg_0001_0005	1534233	CACGACTTTTGCCTGGATTA(A/G) TTCAAATGGCAAAACACCAG	C630_12590	N50S	FAD-dependent oxidoreductase	N
SNP26	sctg_0001_0005	1549284	CAATAGGAATAACATGATGG(C/T) AGTTATCGCATTCAAAAAGT	C630_12670	C34Y	Hypothetical protein	N
SNP27	sctg_0001_0019	44928	CATGGAATCGATCATCAGCC(A/C) TCAACCGCTGGAATATAACC	C630_22780	H479P	Adenylate cyclase	N
SNP28	sctg_0001_0005	698735	ACGCCCGCCCGGCCAGCAAG(G/A) GGCAATACGATTTTCCCTAT	C630_08285	G62R	Methionine aminopeptidase	Y
SNP29	sctg_0001_0019	44922	CGCCGGCATGGAATCGATCA(T/C) CAGCCATCAACCGCTGGAAT	C630_22780	I477T	Adenylate cyclase	N
SNP30	sctg_0001_0025	255355	TTTTGTTTACGGAAGGCTGT(G/A) TGGTAATTCCGAAAAAGGCC	C630_26290/ C630_26295	V27M	Intergenic	Y
SNP31	sctg_0001_0025	255518	TTGTCATTATTTATTCACTG(T/C) AATTGACTCTGTATTCATTT	C630_26290/ C630_26295	V10A	Intergenic	Y
SNP32	sctg_0001_0023	28735	GCCTTTATCGCCATCGTGGT(G/A) CCGCAAATTAAAAGCCAGGC	C630_23355	–	Branched-chain amino acid ABC transporter permease	Y
SNP33	sctg_0001_0021	11112	ACCCGCTGTCTGAGATTACG(C/A) ACAAACGTCGTATCTCCGCA	C630_23090	H526N	DNA-directed RNA polymerase beta subunit	Y
SNP34	sctg_0001_0025	277640	GAACGACGGATCGGCGTTCA(G/A) CTGCGGGTCAAAATGCCACT	C630_26375	–	Glucose dehydrogenase, PQQ-dependent	N
SNP35	sctg_0001_0009	161321	AAGAGTAATCTCTTCGCCCT(C/A) TCCGTCTCGCCCCGGCGAGA	C630_13925/ C630_13930	–	Intergenic	Y
SNP36	sctg_0001_0005	615540	CGCCTCATTTTTGAGAGTGG(G/A) AAATAGAATGGTAGGATAAT	C630_07845/ C630_07850	–	Intergenic	N

Numbering refers to version AMRH02000000.
* Y, yes; N, no.

0 and 21 SNPs. Isolates Kp8 and Kp10 were identical, whilst Kp7 and Kp2 differed from them by two and five non-informative SNPs, respectively. Maximum likelihood phylogenetic analysis of either core genome alignments or concatenated SNP alignments yielded essentially the same tree (Fig. 1). The tree has two major branches that can be identified by three signature SNPs: SNPs 1, 2 and 3 (Table 2). These three SNPs were searched among the genome sequences of ST405 KpO48 isolates from several Spanish hospitals that had been sequenced by the National Reference Laboratory [19]. Of 58 genomes analysed, 48 belonged to the Kp1-3-6 branch (lower branch in Fig. 1) and 10 belonged to the other branch.

A total of 44 additional isolates of ST405 KpO48 were obtained from clinical samples during the study period from 25 different areas (24 hospital wards and primary care). Allele-specific endpoint PCR was used to call the polymorphic variants in these isolates (Table 3). Of the 44 isolates, 9 belonged to the Kp1-3-6 branch and the remaining 35 could be grouped to the other branch. A minimum spanning tree of the 54 isolates was constructed using the phylogenetically informative SNPs. Mapping the isolation date on this tree structure showed the older isolates (2010–2011) in central positions and the newer ones (2012) in terminal positions (Fig. 2).

The isolates were scattered among hospital wards with no evidence of spatial clustering or association between haplotypes and particular units (Table 3). A single isolate was detected in 9 wards, whereas two or more isolates were obtained from each of the remaining 16 wards. Among these, a single haplotype was found in only two wards, whilst two different haplotypes were identified in ten wards and in primary care, and three or more haplotypes were found in three wards (emergency room, a small unit dependent on internal medicine, and a post-surgery recovery section; the three units are often recipients of patients from several different wards).

4. Discussion

In this study, the population structure of ST405 KpO48 collected over 2 years in a single hospital in the context of a sustained outbreak involving several STs was studied. Repetitive extragenic palindromic PCR (rep-PCR) analysis of clonality using the DiversiLab® system suggested that the ST405 KpO48 isolates were a homogeneous group [9], and whole-genome sequencing (WGS) of ten isolates showed that they were indeed very closely related, with 0 to 21 SNPs in pairwise comparisons and seven different SNP profiles. This amount of variation is similar to that observed previously on a broader geographic range [19]. Most (26 of 36) of the SNPs detected involved amino acid substitutions, which may suggest a role for selection in the expansion of the different haplotypes; nevertheless, none of the variants involved proteins related to antigenicity, antimicrobial susceptibility or any other obviously selectable properties (SNP15 in the *ompK36* gene is silent).

SNP calling by allele-specific PCR in a larger set of isolates identified three additional profiles. Despite the limited diversity, more than one-half of the SNPs were phylogenetically informative and exposed a structure within the ST405 KpO48 population. Maximum likelihood phylogeny grouped the sequenced isolates into two major branches. Isolates from the two branches were present in our hospital from the beginning of the outbreak: Kp1 and Kp10 were obtained within 20 days (in December 2010 and January 2011) and differ by four SNPs. Analysis of three signature SNPs indicated that the two branches were already present in several Spanish hospitals in the same period [19], although the relative proportions of the two branches were inverted in that study. This suggests that ST405 KpO48 had been circulating for some months in the population before the first cases were detected. Indeed, a retrospective search in our collection identified one isolate obtained in April 2010 that mapped to the central haplotype in the minimum spanning tree (1670 in Fig. 2). This tree showed a temporal pattern of evolution with sequential haplotype replacements. In contrast, no spatial structure or clustering was detected. There was more than one infection case in 16 of 25 areas, and in 14 of these there were two or more haplotypes. This means that in these 14 areas the infections were not due to the sustained transmission of a single haplotype within the same unit, as might be suggested by lower resolution methods such as MLST or rep-PCR typing, but there was an underlying structure of fast-spreading small outbreaks. Similar findings have been described in other

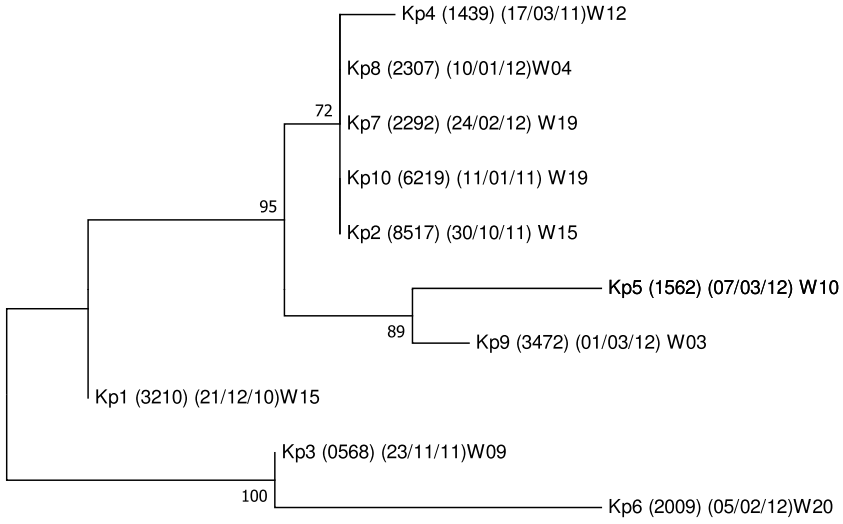

Fig. 1. Molecular phylogenetic analysis of OXA-48-producing *Klebsiella pneumoniae* ST405 sequenced genomes. The evolutionary history was inferred using the maximum likelihood method based on the Tamura–Nei model with MEGA7 [16]. A discrete Gamma distribution was used to model evolutionary rate differences among sites. The tree with the highest log likelihood is shown with bootstrap values indicated next to the branches. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. There were a total of 31 positions in the final data set.

Table 3
Single nucleotide polymorphisms (SNP) profiles of the 54 isolates analysed. The isolates are grouped by similarity. Sample code and isolation date are indicated. The dots indicate sequence positions identical to the first row.

		SNP number																																		
Isolate	Date	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
5475	25-01-2011	A	T	A	A	G	C	C	G	G	C	G	T	A	T	A	A	A	G	A	G	A	T	C	G	A	C	A	G	T	A	T	G	C	G	C	G
0001	07-02-2011	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
0025	18-10-2011	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
0325	16-02-2012	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
5737	28-09-2011	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
1541	02-09-2011	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Kp10	11-01-2011	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
1215	28-12-2010	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
1396	16-03-2011	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
2241	27-01-2012	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
5047	10-01-2011	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
1637	08-02-2011	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
5334	15-11-2012	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
9430	25-01-2011	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Kp8	10-01-2012	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
8678	29-12-2010	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
5728	31-05-2011	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
5869	21-03-2011	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Kp7	24-02-2012	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	C	.	C	.	.	.	.	.	.	.
5383	27-06-2011	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Kp2	30-10-2011	.	.	.	T	.	.	.	A	A	.	.	.	.	.	G	.	.	.	G	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.
1077	28-12-2012	.	.	.	.	.	.	.	.	.	G	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
3278	27-09-2012	.	.	.	.	.	.	.	.	.	G	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
5173	07-11-2012	.	.	.	.	.	.	.	.	.	G	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
2661	30-06-2011	.	.	.	.	.	.	.	.	.	G	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
5202	08-11-2012	.	.	.	.	.	.	.	.	.	G	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
3781	22-10-2012	.	.	.	.	.	.	.	.	.	G	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
2837	10-09-2012	.	.	.	.	.	.	.	.	.	G	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
5364	16-11-2012	.	.	.	.	.	.	.	.	.	G	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
2734	03-09-2012	.	.	.	.	.	.	.	.	.	G	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
5834	26-09-2013	.	.	.	.	.	.	.	.	.	G	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
3771	22-10-2012	.	.	.	.	.	.	.	.	.	G	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
8436	04-06-2012	.	.	.	.	.	.	.	.	.	G	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Kp4	17-03-2011	.	.	.	.	.	.	.	.	.	G	.	.	T	.	G	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.
1670	29-04-2010	.	.	.	.	T	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
0789	23-02-2011	.	.	.	.	T	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
7823	21-01-2011	.	.	.	.	T	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
7106	29-07-2011	.	.	.	.	T	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	A	A	.	.	.
8535	07-11-2011	.	.	.	.	T	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Kp9	01-03-2012	.	.	.	.	T	.	.	.	.	.	.	A	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	A	A	A	.	.
0104	04-05-2012	.	.	.	.	T	.	.	.	.	.	A	.	.	C	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	A	.	.	.	.
Kp5	07-03-2012	.	.	.	.	T	.	.	.	.	.	A	.	.	C	.	.	.	.	.	A	C	.	.	T	.	.	.	.	.	C	.	A	.	.	.	A
3212	16-04-2012	T	G	T	.	T	A	T	.	.	.	.	.	.	.	G	T	T	A	.	.	.	G	G	.	.	.	.	A	.	.	C	.	.	.	A	.
Kp6	05-02-2012	T	G	T	.	T	A	T	.	.	.	.	.	.	.	G	T	T	A	.	.	.	G	G	.	.	.	.	A	.	.	C	.	.	.	A	.
9799	12-04-2012	T	G	T	.	T	A	T	.	.	.	.	.	.	.	G	T	T	A	.	.	.	G	G	.	.	.	.	A	.	.	C	.	.	.	A	.
6286	15-12-2012	T	G	T	.	T	A	T	.	.	.	.	.	.	.	G	T	T	A	.	.	.	G	G	.	.	.	.	A	.	.	C	.	.	.	A	.
5895	29-10-2012	T	G	T	.	T	A	T	.	.	.	.	.	.	.	G	T	T	A	.	.	.	G	G	.	.	.	.	A	.	.	C	.	.	.	A	.
0800	23-02-2011	T	G	T	.	T	A	T	.	.	.	.	.	.	.	G	T	T	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.
5761	02-02-2011	T	G	T	.	T	A	T	.	.	.	.	.	.	.	G	T	T	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.
5914	24-10-2011	T	G	T	.	T	A	T	.	.	.	.	.	.	.	G	T	T	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.
4926	05-08-2011	T	G	T	.	T	A	T	.	.	.	.	.	.	.	G	T	T	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.
Kp3	23-11-2011	T	G	T	.	T	A	T	.	.	.	.	.	.	.	G	T	T	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.
Kp1	21-12-2010	T	G	T	.	T	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
1546	22-03-2011	T	G	T	.	T	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

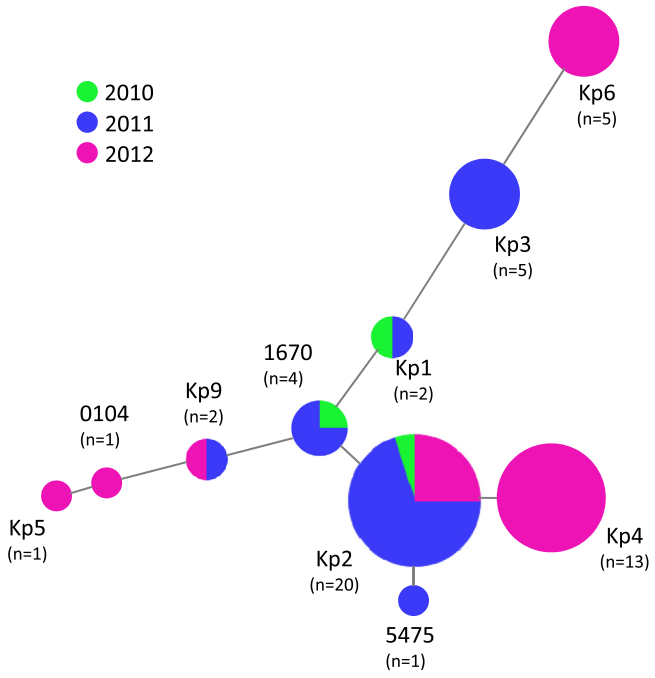

Fig. 2. Population structure of OXA-48-producing *Klebsiella pneumoniae* (KpO48) ST405 isolates from our hospital. Minimum spanning tree of the single nucleotide polymorphism (SNP) haplotypes of 54 clinical isolates of ST405 KpO48. Circles are proportional to the number of isolates with the same SNP haplotype. Haplotypes are arbitrarily named after one isolate and are coloured according to the year of isolation. The distances between circles are proportional to the number of SNPs between haplotypes.

studies, underlining the value of WGS to reliably track pathogen outbreaks [20,21].

A limitation of the allele-specific PCR approach is that it does not show the full genome variability in the whole population, only that previously identified in the sequenced isolates, but it is a fast and simple approach and the definition of signature SNPs might be useful for the rapid analysis of tens to hundreds of isolates. In addition, the study was limited to isolates obtained from clinical samples because patient and environmental surveillance criteria changed as the outbreak developed.

We have characterised the population structure of ST405 KpO48 during the first 2 years of an outbreak. We have found no evidence of spatial clustering, with up to three independently evolving lineages, which suggest that the reservoir of ST405 KpO48 during the study period was a large population of colonised patients and the outbreak was composed of a series of small outbreaks.

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Competing interests

None declared.

Ethical approval

Not required.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.jgar.2018.06.008.

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