Diversity of Capsular Polysaccharide Gene Clusters in Kpc-Producing Klebsiella pneumoniae Clinical Isolates of Sequence Type 258 Involved in the Italian Epidemic

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Abstract

Strains of Klebsiella pneumoniae producing KPC-type beta-lactamases (KPC-Kp) are broadly disseminating worldwide and constitute a major healthcare threat given their extensively drug resistant phenotypes and ability to rapidly disseminate in healthcare settings. In this work we report on the characterization of two different capsular polysaccharide (CPS) gene clusters, named cpsBO-4 and cps207-2, from two KPC-Kp clinical strains from Italy belonging in sequence type (ST) 258, which is one of the most successful ST of KPC-Kp spreading worldwide. While cpsBO-4 was different from known 78 K-types according to the recently proposed typing schemes based on the wzi or wzc gene sequences, cps207-2 was classified as K41 by one of these methods. Bioinformatic analysis revealed that they were represented in the genomic sequences of KPC-Kp from strains of ST258 from different countries, and cpsBO-4 was also detected in a KPC-Kp strain of ST442 from Brazil. Investigation of a collection of 46 ST258 and ST512 (a single locus variant of ST250) clinical strains representative of the recent Italian epidemic of KPC-Kp by means of a multiplex PCR typing approach revealed that cpsBO-4 was the most prevalent type, being detected both in ST258 and ST512 strains with a countrywide distribution, while cps207-2 was only detected in ST258 strains with a more restricted distribution.

Introduction

The capsular polysaccharide (CPS or K-antigen) is a recognized virulence factor of Klebsiella pneumoniae [1,2]. This component exhibits a remarkable intra-specific structural diversity which translates into different antigenic properties that may be relevant to bacterial virulence [2–4]. CPS diversity has classically been detected by serotyping techniques [3], but genotyping systems have recently been developed, offering several advantages vs. the conventional serotyping approach [6–10]. Among systems that do not require a sequencing step, a PCR-based typing system has been proposed for the detection of isolates of the K1, K2, K5, K20, K54 and K57 capsular types, that are commonly associated with invasive diseases or having a prominent pathogenicity [6]. Conversely, two systems based on amplification and sequencing of the conserved wzi and wzc genes were recently proposed to determine the K-type of K. pneumoniae [9,10].

During the last years, strains of K. pneumoniae producing KPC-type carbapenemases (KPC-Kp) belonging in sequence type (ST) 258 and related variants (e.g. ST312, ST437 and ST11) have undergone a global dissemination, with epidemic diffusion in some areas of North and South America, Europe and Asia [11–18]. Infections caused by these strains pose a major challenge due to their extended antibiotic resistance phenotypes and ability to rapidly disseminate in healthcare settings, and are associated with high mortality rates [19–20]. Detailed knowledge on the CPSs of these strains, however, is still limited. A ST258 KPC-Kp strain from Greece has recently been reported to express a K41 serotype CPS [21], while the chemical structure of the CPS of two representatives of an outbreak clone of ST258 KPC-Kp from USA has recently been described [22].

In this work we have characterized two different cps gene clusters from two KPC-Kp clinical strains of ST258 from Italy, and report on their distribution in a collection of KPC-Kp isolates of ST258 and ST512 representative of the recent Italian epidemic. We also propose a modification to a previously established PCR-based CPS typing system [6], to include recognition of these CPS types.

Results

Characterization of Two Different CPS Gene Clusters in ST258 KPC-Kp Strains of Clinical Origin

The CPS gene cluster of two KPC-Kp strains of clinical origin, KKBO-4 and KK207-2, were characterized by an HTGS
approach. The two strains had been isolated in 2010 from bloodstream infections of inpatients in two different Italian hospitals and produced either KPC-2 (KK207-2) or KPC-3 (KKBO-4). They were both of ST238, and exhibited a related although not identical XbaI PFGE profile [12] (difference of two bands, data not shown).

Comparison of the draft genomes using GGDC 2.0 confirmed the close relatedness between the two strains at the genomic level (intergenic distance of 0.0015). Despite this close relatedness, however, the _cps_ gene clusters of the two strains were significantly different from each other.

The CPS gene cluster of KKBO-4 (named _cpsBo-4_) was found to be 26,587 bp-long, consisting of 20 ORFs (from _galF_ to _wzy_), and was characterized by the presence of the K-antigen flippase- and polymerase-encoding genes (wzy and _wcaJ_, respectively) at the 3’-end, and by the presence of the _mlBADC_ operon for the synthesis of dTDP-L-rhamnose in the central region (Fig. 1).

The _cpsBo-4_ gene cluster was identical or very similar to those present in a number of ST258 _K. pneumoniae_ strains from different countries, whose genome sequences are available in the public domain, and also very similar to that previously described in a ST142 KPC-Kp strain (Kp13) that caused an outbreak in Brazil [23] (Table 1 and Fig. 1). Compared to the CPS gene cluster of _K. pneumoniae_ HS11286 (ST11, a single locus variant of ST258) [29], _cpsBo-4_ exhibited significant similarities in some regions (e.g., from _galF_ to _orf8_ and from _gnd_ to _wzc_1, comprising the _mlBADC_ operon), but also substantial differences in the central and the 3’-region of the gene cluster (Fig.1).

According to the _eps_-typing protocol based on sequencing of the _wzi_ gene [9] _epsBo-4_ showed a single nucleotide difference with the _wzi81-K81_ reference amplicon. According to the _eps_-typing protocol based on sequencing of the _wzi_ gene [10] _epsBo-4_ was <80% identical to that of any other reference sequence.

The CPS gene cluster of strain KK207-2 (named _cps207-2_) was found to be 23,994 bp-long, consisting of 19 ORFs (from _galF_ to _ugd_). It did not contain the _mlBADC_ operon but contained original genes, of which some encode putative glycosyltransferases, located between the _wzy_ and _wcaJ_ genes (Fig. 1). The _cps207-2_ gene cluster was very similar to those present in ST238 _K. pneumoniae_ strains from USA, whose genome sequences are available in the public domain (Table 1). It also exhibited regions of similarity with the CPS gene clusters of _K. pneumoniae_ strains 1996/49 and 8238, producing CPS of the K22 and K37 serotype, respectively, and with both _cpsBo-4_ and _cps11286_ (Fig. 1).

According to the _eps_-typing protocol based on sequencing of the _wzi_ gene [9] _eps207-2_ was identical to the _wzi29-K41_ reference amplicon. According to the _eps_-typing protocol based on sequencing of the _wzi_ gene [10] _eps207-2_ was 93% identical to the _K22_ref_ and _K37_ref_ reference sequences.

Taken together, these results suggested that the CPS composition of KK207-2 was different from that of KKBO-4, demonstrating that at least two different types of CPS gene clusters may be found in KPC-Kp of ST238, and that _cpsBo-4_-like gene clusters can also be found in KPC-Kp of unrelated STs such as ST442.

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**Figure 1. Comparison of the CPS gene clusters from _K. pneumoniae_ strains KKBO-4 (cpsBo-4), HS11286 (cpsHS11286), KK207-2 (cps207-2), and 1996/49 (K-type 22, cpsK22).** Sequence accession numbers and STs for the respective strains are also indicated (the ST of strain 1996/49 was deduced from ref. 30). The CPS gene cluster of strain 8238 (K-type 37) (accession number AB819894), differing from _cpsK22_ by a single nucleotide deletion resulting in a frameshift mutation located in a putative acetyltransferase downstream _gnd_, is not included for simplicity. Homologous regions are connected by areas of different colors reflecting the degree of nucleotide identity (from 67% to 100%). Open reading frames encoding transposases are colored in red, while those encoding hypothetical glycosyltransferases are colored in yellow. The locations of synonymous, non-synonymous and intergenic single nucleotide variations (SNVs) occurring between the CPS gene clusters of KKBO-4 and Kp13 are indicated by green, red and black stars, respectively. The _cps207-2_ gene cluster exhibited regions of similarity to _cpsBo-4_ including the conserved _galF-wzc_ region (83.2% of nucleotide identity), and the conserved _gnd_ and _ugd_ genes (95.5% and 96.8% nucleotide identity, respectively).

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Analysis of the cps Gene Clusters in a Contemporary Collection of ST258 and ST512 KPC-Kp Strains from the Italian Epidemic

A multiplex PCR protocol derived from that originally proposed by Turton et al. [6], modified to detect the cpsBO-4 and cps207-2 gene clusters, was used to analyze a collection of 46 nonreplicate KPC-Kp clinical strains of ST258 or ST512 isolated from 19 different centers (Fig. 2) during the first Italian countrywide survey on carbapenem-resistant Enterobacteriaceae [12] and selected as representatives of the recent Italian KPC-Kp epidemic. Nine additional carbapenem-resistant but KPC-negative K. pneumoniae strains with different carbapenem-resistance mechanisms (production of VIM-1, of OXA-48, or of an extended-spectrum beta-lactamase in presence of a permeability defect), isolated during the same survey, were also analyzed for comparison.

Of the 46 KPC-Kp strains, 38 (82.6%) carried a cpsBO-4-like cluster, while the remaining 8 (17.4%) carried a cps207-2-like cluster (Table 2). The cpsBO-4-like gene cluster was detected in both ST258 and ST512 strains from all 19 centers, while the cps207-2-like gene cluster was only detected in ST258 strains from 4 centers (Table 2, Fig. 2). The 9 KPC-negative strains were not typeable by the modified multiplex PCR, with the exception of one isolate identified as K2, indicating that none of those strains carried cpsBO-4 or cps207-2-like gene clusters. According to the wzi sequence-based typing method [9], the isolates were genotyped as K2 (n = 1), K9 (n = 1), K17 (n = 3), K38 (n = 1), K14/64 (n = 1), K15/17/50/51/52 (n = 2). Results were consistent with the fact that none of these K-types, except K2, could be detected by the modified multiplex PCR method.

Discussion

Results of this work showed that KPC-Kp belonging to ST258, which have largely contributed to the epidemic dissemination of

### Table 1. Differences between the CPS gene clusters of strains KKBO-4 (cpsBO-4, 26,587 bp) or KK207-2 (cps207-2, 23,994 bp) and closely related CPS gene clusters detected in other sequenced genomes.

| Strains   | STs | Countries |cps-types| Nucleotide Differences | Gene Mutations (AminoAcid Differences) | References/Accession numbers |
|-----------|-----|-----------|---------|-----------------------|---------------------------------------|------------------------------|
| KPNH21    | 258 | USA       | cpsBO-4 | -                     | -                                     | [24]                         |
| ST258-490 | 258 | Israel    | cpsBO-4 | -                     | -                                     | [25]                         |
| ST258 K2680 | 258 | Italy     | cpsBO-4 | -                     | -                                     | [26]                         |
| MP14      | 258 | South Korea| cpsBO-4 | -                     | -                                     | [27]                         |
| ATCC BAA-1705 | 258 | USA     | cpsBO-4 | 1                     | galf a191c (E64A)                     | [28]                         |
| UHKPC02   | 258 | USA       | cpsBO-4 | 1                     | galf a191c (E64A) ARSK00000000.1      |                              |
| BIDMC 12C | 258 | USA       | cpsBO-4 | 2                     | galf a191c (E64A) ARXLG00000000.1     |                              |
| Kp13      | 442 | Brazil    | cpsBO-4 | 5d                    | galf T366a (silent)                    | [23]                         |
| UHKPC06   | 258 | USA       | cps207-2| 1                     | wzc c1936a (P646T) ARJS00000000.1     |                              |

The dash indicates 100% identity. The cut-off values used for the inclusion in the analysis were ≥99% nucleotide identity and ≥99% of query coverage, based on results from a BLAST search performed at the NCBI website (http://blast.ncbi.nlm.nih.gov/) using either nr or wgs databases, using default values but without the low complexity filter option.

*KPNH21 was chosen as a representative of the outbreak clone described in reference 24.

†Strains ST258 K2680 and ST258 K2880, both described in reference 26, were characterized by identical CPS gene clusters.

‡Strains UHKPC02 and UHKPC06 were representatives of those included in the Klebsiella pneumoniae Genome Sequencing Center Project (http://gsc.jcvi.org/projects/gscklebsiella_pneumoniae/index.php).

§2 out of 5 SNVs are located in intergenic regions.

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Figure 2. Map showing the distribution of Italian centers from which the 46 KPC-Kp strains of ST258 or ST512 investigated for CPS typing by the modified multiplex PCR were originated, and distribution of the different types of cps gene clusters. Centers were as follows: 01, Milan; 02, Varese; 03, Lecco; 04, Torino; 05, Novara; 06, Genoa; 07, Sanremo; 08, Verona; 09, Bolzano; 10, Modena; 11, Modena; 15, Ancona; 16, Rome; 18, Foggia; 19, Lecce; 20, Naples; 22, Cosenza; 23, Palermo; 24, Catania.

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Table 2. KPC-Kp strains of clinical origin from the Italian nationwide survey investigated for the nature of the *cps* gene cluster by the modified multiplex PCR developed in this work.

| Strain ID | Sample          | PFGE | ST  | *cps*-type |
|-----------|-----------------|------|-----|------------|
| 01C03     | Urine           | A6   | 258 | *cps*207-2 |
| 01C06     | Urine           | A3   | 258 | *cps*207-2 |
| 01C08     | Wound exudate   | A6   | 258 | *cps*207-2 |
| 01C22     | Wound exudate   | A0   | 512 | *cps*BO-4  |
| 02C01     | Urine           | A5   | 512 | *cps*BO-4  |
| 02C06     | Wound exudate   | A2   | 258 | *cps*BO-4  |
| 03C06     | Wound exudate   | A4   | 512 | *cps*BO-4  |
| 03C08     | Urine           | A3   | 258 | *cps*207-2 |
| 03C12     | Urine           | A1   | 258 | *cps*207-2 |
| 04C35     | Urine           | A0   | 512 | *cps*BO-4  |
| 04C38     | Bronchial aspirate | A4 | 512 | *cps*BO-4  |
| 04C49     | Bronchial aspirate | A2 | 258 | *cps*BO-4  |
| 05C15     | Blood           | A4   | 512 | *cps*BO-4  |
| 06C02     | Urine           | A3   | 258 | *cps*207-2 |
| 06C04     | Urine           | A4   | 512 | *cps*BO-4  |
| 06C05     | Urine           | A2   | 258 | *cps*BO-4  |
| 06C07     | Bronchial aspirate | A3 | 258 | *cps*207-2 |
| 06C19     | Blood           | A0   | 512 | *cps*BO-4  |
| 07C06     | Urine           | A3   | 258 | *cps*BO-4  |
| 07C07     | Bronchial aspirate | A2 | 258 | *cps*207-2 |
| 08C02     | Urine           | A0   | 512 | *cps*BO-4  |
| 08C04     | Urine           | A2   | 258 | *cps*BO-4  |
| 09C06     | Urine           | A1   | 258 | *cps*BO-4  |
| 10C04     | Urine           | A5   | 512 | *cps*BO-4  |
| 10C09     | Wound exudate   | A4   | 512 | *cps*BO-4  |
| 11C07     | Urine           | A5   | 512 | *cps*BO-4  |
| 15C05     | Bronchial aspirate | A4 | 512 | *cps*BO-4  |
| 15C10     | Urine           | A2   | 258 | *cps*BO-4  |
| 15C15     | Bronchial aspirate | A0 | 512 | *cps*BO-4  |
| 15C18     | Urine           | A1   | 258 | *cps*BO-4  |
| 16C05     | Blood           | A4   | 512 | *cps*BO-4  |
| 16C12     | Wound exudate   | A2   | 258 | *cps*BO-4  |
| 18C01     | Bronchial aspirate | A4 | 512 | *cps*BO-4  |
| 18C22     | Abscess         | A2   | 258 | *cps*BO-4  |
| 18C24     | Wound exudate   | A1   | 258 | *cps*BO-4  |
| 19C09     | Blood           | A5   | 512 | *cps*BO-4  |
| 19C11     | Blood           | A2   | 258 | *cps*BO-4  |
| 20C14     | Blood           | A6   | 258 | *cps*BO-4  |
| 22C06     | Bronchial aspirate | A2 | 258 | *cps*BO-4  |
| 22C09     | Urine           | A1   | 258 | *cps*BO-4  |
| 22C24     | Wound exudate   | A4   | 512 | *cps*BO-4  |
| 23C10     | Urine           | A1   | 258 | *cps*BO-4  |
| 23C13     | Bile            | A6   | 258 | *cps*BO-4  |
| 24C02     | Blood           | A2   | 258 | *cps*BO-4  |
| 24C20     | Bronchial aspirate | A2 | 258 | *cps*BO-4  |
| 24C21     | Bronchial aspirate | A2 | 258 | *cps*BO-4  |

The first two characters of each strain ID identify the center from which the isolate was obtained. Identifiers are as reported in the legend to Fig. 2.

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KPC-Producing K. pneumoniae ST258 Capsular Gene Clusters

the KPC-type beta-lactamases in Italy and elsewhere [12,20], can be equipped with at least two different types of CPS gene clusters, here named \( \text{cps} \)BO-4 and \( \text{cps} \)207-2. The former type was more prevalent in a collection of representative isolates from the recent Italian epidemic, being also present in strains of ST512. The differences in the nature of these two CPS gene clusters could be related with differences in the ability of spreading and virulence of different clones, which will deserve further investigation.

The detailed chemical structure of \( \text{cps} \)BO-4 was recently solved for two representatives of the outbreak clone of KPC-Kp found at the Clinical Center of the U.S. National Institutes of Health [22,24]. The Authors demonstrated that this CPS type is structurally different from any other published \( K. \) pneumoniae CPS, even if similarity to \( K. \) pneumoniae K19 and K34 antigens was observed, possibly explaining the cross-reactivity of this CPS with \( K. \) pneumoniae even if structurally different from any other published.

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On the other hand, results obtained for \( \text{cps} \)207-2 using the above genotyping methods were not in agreement between each other. In fact, while according to the \( wzi \)-based method [10] \( \text{cps} \)207-2 corresponded to a new K-type, according to the \( wzi \)-based method [9] this gene cluster corresponds to the known K41 K-type. The result obtained with the \( wzi \)-based method could be consistent with the finding that a strain of KPC-Kp of ST258, representative of the dominant clone circulating in Greece during 2009–2011, was serotyped as K41 [21]. This finding also suggests that this K-type has achieved a significant distribution in some settings, and it would therefore be interesting to further investigate the nature of the whole CPS gene cluster in KPC-Kp strains of K-type 41.

Data presented here also confirmed that the CPS gene cluster do not unambiguously correlate with any particular ST, confirming the notion that CPS gene clusters can be exchanged between different strains of \( Enterobacteriaceae \) species [30–32].

Materials and Methods

Bacterial Strains

Two KPC-Kp strains of ST258, KKBO-4 and KK207-2, isolated in 2010 from two different Italian hospitals and epidemiologically unrelated with each other, were used for high-throughput genome sequencing (HTGS) analysis and characterization of their \( \text{cps} \) gene clusters.

Forty-six additional KPC-Kp strains of ST258 or ST512 plus nine carbapenem-resistant but KPC-negative \( K. \) pneumoniae strains of different STs were investigated by the modified multiplex PCR for CPS genotyping developed in this work. These strains were selected as representative of the recent Italian epidemic of carbapenem-resistant \( K. \) pneumoniae from a collection of clinical isolates obtained during the first nationwide survey on carbapenem-resistant \( K. \) pneumoniae strains. Extraordinary spread of \( K. \) pneumoniae of ST258 was reported in March 2010 in Italy [12,19].

High-Throughput Genome Sequencing and Analysis of Sequence Data

HTGS was performed using a HiSeq 2000 Illumina platform and a paired-ends protocol with an average insert size of 300 bp.

Reads were assembled using ABySS [33]. GGDC software was used to assess the genomic diversity of the investigated isolates [34]. HTGS for strain KKBO-4 has been described previously [35]. The web interface of BLAST available at the NCBI website was used to compare the CPS gene clusters of the two strains with homologues in the nr or wgs databases [36]. CPS gene clusters sequences were aligned with ClustalX [37]. Structural comparisons of KKBO-4, KK207-2 and other published \( \text{cps} \) gene clusters were performed with EasyFig [39].

The nucleotide sequences of the \( \text{cps} \) gene clusters of KKBO-4 and KK207-2 were deposited in the DDBJ/EMBL/GenBank databases under accession numbers HE866751 and HE866752, respectively.

Multiplex PCR for CPS Typing

CPS gene clusters were genotyped using a multiplex PCR approach as previously described [6], modified by including two additional primer pairs designed to amplify specific targets in the \( \text{cps} \) gene clusters described in this paper: \( \text{wzi} \)BO-4F (5’-CGGTTTCTGATGCAAGGG-3’) and \( \text{wzi} \)BO-4R (5’-ATCATGTGTCATTCCAAGGATAC-3’), targeting the \( \text{wzi} \) gene of the \( \text{cps} \)BO-4 gene cluster, and hgt207-2F (5’-GGCTGTTATTTCCGC-3’) and hgt207-2R (5’-CATATGCTCATAAAC-CCGCC-3’), targeting a hypothetical glycosyltransferase gene of the \( \text{cps} \)207-2 gene cluster [40]. These two additional primer pairs yielded amplicons of 478 and 352 bp, being suitable for the inclusion in the multiplex PCR because of the unique band sizes. Primers \( K. \) pneumoniae Pf and \( K. \) pneumoniae Pr1, designed for the identification at the species level of \( K. \) pneumoniae and included in the original multiplex PCR protocol, were not included in the reaction mix.

Addendum in Proof

After the revised version of this manuscript had been submitted, two articles have been published reporting the occurrence of two distinct \( \text{cps} \) gene clusters in \( Klebsiella \) pneumoniae isolates belonging to the ST258 clonal lineage [39], and the development of a PCR-based assay for their detection [40]. The two gene clusters, named \( \text{cps} \)-1 and \( \text{cps} \)-2, correspond to \( \text{cps} \)207-2 and \( \text{cps} \)BO-4 described here, respectively, while the PCR assay targets the different \( wzi \) genes of the two clusters. At the same time, an additional article has been published reporting that \( K. \) pneumoniae isolates of ST258 are characterized by \( \text{cps} \) gene clusters carrying a novel \( wzi \) allele (\( wzi \)-154) [41], that is identical to the \( wzi \) allelic variant of \( \text{cps} \)BO-4.

Author Contributions

Conceived and designed the experiments: MMD FA GMR. Performed the experiments: MMD FA TG VC NC. Analyzed the data: MMD FA TG LS. Wrote the paper: MMD GMR. Provided the clinical isolates, including associated clinical data, and participated in the critical discussion of results: SA LS.

PREFERENCES

1. Podschun R, Ullmann U (1998) \( Klebsiella \) spp. as nosocomial pathogens: epidemiology, taxonomy, typing methods, and pathogenicity factors. Clin Microbiol Rev 11: 589–603.

2. Cortés G, Borrell N, de Astorza B, Gómez C, Saula de la Fuente J, et al. (2002) Molecular analysis of the contribution of the capsular polysaccharide and the lipopolysaccharide O side chain to the virulence of \( Klebsiella \) pneumoniae in a murine model of pneumonia. Infect Immun 70: 2583–2590.

3. Shu HY, Fang CP, Liu YM, Wu KM, Chen YT, et al. (2009) Genetic diversity of capsular polysaccharide biosynthesis in \( Klebsiella \) pneumoniae clinical isolates. Microbiology 155: 4170–4183.
4. Mizuta K, Ohta M, Mori M, Hasegawa T, Nakashima L, et al. (1983) Virulence for mice of K. pneumoniae strains belonging to the O1 group: relationship to their capsular (K) types. Infect Immun 40: 56–61.

5. Orskov I, Orskov F (1984) Serotyping of Klebsiella. In: “Methods in Microbiology” (T Bergan, ed). Academic Press, London. 143–164.

6. Turton JF, Perry C, Egeloh S, Hampton CV. (2010) PCR characterization and typing of K. pneumoniae using capsular type-specific, variable number tandem repeat and virulence gene targets. J Med Microbiol 59: 541–547.

7. Brisse S, Isen Guth-Jeanjean S, Grimmel PA (2004) Molecular serotyping of Klebsiella species isolates by restriction of the amplified capsular antigen gene cluster. J Clin Microbiol 42: 3388–3390.

8. Turton JF, Baklan H, Sun J, Kaufmann ME, Pitt TL (2008) Evaluation of a multiplex PCR for detection of serotypes K1, K2 and K5 in Klebsiella sp. and comparison of isolates within these serotypes. FEMS Microbiol Lett 284: 247–252.

9. Brisse S, Passet V, Haugard AB, Babosan A, Kassis-Chikhani N, et al. (2013) wz2 gene sequencing, a rapid method for determination of capsular type for K. pneumoniae. J Clin Microbiol 51: 4073–4078.

10. Pan YJ, Lin TL, Chen YH, Hsu CR, Hsieh PF, et al. (2013) Capsular types of K. pneumoniae revisited by w2 Sequencing. PLoS ONE 8(12): e80670.

11. Nordmann P, Naas T, Poirel L. (2011) Global spread of carbapenemase-producing Enterobacteriaceae. Emerg Infect Dis 17: 1791–1798.

12. Giani T, Pini B, Arena F, Conte V, Bracco S, et al. (2013) Epidemic diffusion of KPC carbapenemase-producing K. pneumoniae in Italy: results of the first countrywide survey, 15 May to 30 June 2011. Euro Surveill 16.

13. Qi Y, Wei Z, Ji S, Du X, Shen P, et al. (2011) ST11, the dominant clone of KPC-producing K. pneumoniae in China. J Antimicrob Chemother 66: 307–312.

14. Li J, Sheng ZK, Deng M, Bi S, Hu FS, et al. (2012) Epidemic of K. pneumoniae ST11 clone coproducing KPC-2 and 16S rRNA methylase RmtB in a Chinese University Hospital. BMC Infect Dis 12: 373.

15. Yang J, Ye Y, Guo L, Zhao Q, Chen R, et al. (2013) A nosocomial outbreak of KPC-2-producing K. pneumoniae in a Chinese hospital: dissemination of ST11 and emergence of ST37, ST392 and ST395. Clin Microbiol Infect 19: 1520–1524.

16. Lauderdale TL, Shi ZY, Lin CF, Lai JF, Tan MC, et al. (2013) ABySS: a parallel assembler for short read sequence data. Genome Res 19: 1117–1123.

17. Andrade LN, Curiao T, Ferreira JC, Longo JM, Climaco EC, et al. (2011) ST11, the dominant clone of KPC-producing K. pneumoniae strains available from the American Type Culture Collection. Genome Announce 1. 130: e00112–13 [pii].10.1128/genomeA.

18. Hong SK, Yong D, Kim K, Hong SS, Hong SG, et al. (2013) First outbreak of KPC-producing K. pneumoniae sequence type 236 in a hospital in South Korea. J Clin Microbiol 51: 3877–3879.

19. Tzouvelekis LS, Markogiannakis A, Psichogiou M, Tassios PT, Daikos GL. (2010) Virulent clones of K. pneumoniae: identification and evolutionary scenario based on genomic and phenotypic characterization. PLoS One 4: e8492.

20. Nelson K, Salender RK (1994) Intergeneric transfer and recombination of the 6-phosphogluconate dehydrogenase gene (pgd) in enteric bacteria. Proc Natl Acad Sci U S A 91: 10227–10231.

21. KPC-Producing K. pneumoniae ST258 epidemic strain. J Bacteriol 194: 1101–1102.

22. Brisse S, Passet V, Isen Guth-Jeanjean S, Tournebize R, et al. (2009) Virulent clones of K. pneumoniae: identification and evolutionary scenario based on genomic and phenotypic characterization. PLoS One 4: e8492.

23. Chmelnitsky I, Doniger T, Shklyar M, Naparstek L, Banin E, et al. (2009) ABySS: a parallel assembler for short read sequence data. Genome Res 19: 1117–1123.

24. ABySS: a parallel assembler for short read sequence data. Genome Res 19: 1117–1123.

25. Snitkin ES, Zelazny AM, Thomas PJ, Stock F, Henderson DK, et al. (2012) Tracking a hospital outbreak of carbapenem-resistant K. pneumoniae with whole-genome sequencing. Sci Transl Med 4: 148ra116.

26. Chmelnitsky I, Doniger T, Shklyar M, Naparstek L, Banin E, et al. (2012) Draft genome sequence of an extremely drug-resistant KPC-producing K. pneumoniae ST258 epidemic strain. J Bacteriol 194: 6014.

27. Cannatelli A, D’Andrea MM, Giani T, Di Pilato V, Arena F, et al. (2013) Capsular types of K. pneumoniae ST258 strains. Antimicrob Agents Chemother Apr 14. [Epub ahead of print].

28. Snitkin ES, Zelazny AM, Thomas PJ, Stock F, Henderson DK, et al. (2012) Tracking a hospital outbreak of carbapenem-resistant K. pneumoniae with whole-genome sequencing. Sci Transl Med 4: 148ra116.

29. Cannatelli A, D’Andrea MM, Giani T, Di Pilato V, Arena F, et al. (2013) First outbreak of KPC-2-producing K. pneumoniae sequence type 236 in a hospital in South Korea. J Clin Microbiol 51: 3877–3879.

30. Chmelnitsky I, Doniger T, Shklyar M, Naparstek L, Banin E, et al. (2012) Draft genome sequences of two multidrug-resistant K. pneumoniae ST258 isolates resistant to colistin. Genome Announce 1. 130: e00112–13 [pii].10.1128/genomeA.

31. Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, et al. (2007) Clustal W and Clustal X version 2.0. Bioinformatics 23: 2947–2948.

32. Sullivan MJ, Petty NK, Beaton SA (2011) Easyfig: a genome comparison visualizer. Bioinformatics 27: 1490–1491.

33. Diagbe-Navarro E, Chen L, Passet V, Burak S, Ulica-Hernando A, et al. (2014) Carbapenem-resistant K. pneumoniae exhibit variability in capsular polysaccharide and capsule associated virulence traits. J Infect Dis. Mar 14. [Epub ahead of print].