**Supplemental materials**

Table S1. Primers used in this study.

Table S2. List of KPC alleles with insertions at Ambler position 269, available in the NCBI database (April 2024).

**Table S1. Primers used in this study.**

|  |  |  |
| --- | --- | --- |
| Primersa | Sequence (5′-3′) | Use for |
| KPC\_NdeI\_F | AAAAACATATGGTCATCCGCAGACCAACG | *bla*kpc cloning |
| KPC\_EcoRI\_R | CCGGAATTCCAGACTCCTAGCCTAAATGTGA |
| KPC-F | GACACACCCATCCGTTAC | *bla*kpc detection |
| KPC-R | CCAACTCCTTCAGCAACA |
| J53\_F | ACGGACTAACAGCCTGGAAA | confirming J53 for conjugation |
| J53\_R | TAGCGTATCCAGCGTCACTT |
| T7 | TAATACGACTCACTATAGGG | confirming cloning fragment |
| T7ter | TGCTAGTTATTGCTCAGCGG |
| IncFII\_F | AGAAGGAGTGAGCACAGA | replicon confirming |
| IncFII\_R | TTGGCGAGTCAGAAAGATT |
| KPC\_frg\_F | tgtattttcagggtgctagcCTGACCAACCTCGTCGCG | *bla*KPC gene sequence (residues 25-293) |
| KPC\_frg\_R | tggtggtggtggtgctcgagTTACTGCCCGTTGACGCC |
| KPC\_vec\_F | CTCGAGCACCACCACCACC | the pET-28a vector |
| KPC\_vec\_R | GCTAGCACCCTGAAAATACAGATTT |

aAll Primers except the universal T7 and T7ter were self-designed. Restriction sites are underlined

**Table S2. List of KPC alleles with insertions at Ambler position 269, available in the NCBI database (April 2024).**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **KPC-varianta** | **Accession Number** | **Divergence from** | **Omega loop** | **Loop 266-275** | **CZA treated** | **MICs (mg/L)**b |  | **PMID** | **References** |
| **CZA** | **MEM** |
| KPC-204 | OR979533 | KPC-2 |  | ins\_269\_KDD | No | 128 | 16 |  |  |
| KPC-29 | AY034847 | KPC-3 |  | ins\_269\_KDD | Yes | 24 | 16 | 34339281 | [1] |
| KPC-58\* | MT463289 | KPC-2 |  | ins\_269\_KDDNRAPN |  |  |  |  |  |
| KPC-134\* | OP293349 | KPC-2 | D179A | ins\_269\_KDDNRAPN |  |  |  |  |  |
| KPC-93 | MZ569034 | KPC-2 |  | ins\_269\_NRAPN | Yes | 64 | 0.06 | 35416703 | [2] |
| KPC-205\* | JAYEEW010000028 | KPC-3 |  | ins\_269\_NRAPN |  |  |  |  |  |
| KPC-76 | MT550690 | KPC-2 | D179Y | ins\_269\_VYTRAPN | Yes | 16 | ≤0.03 | 34935416,35588280 | [3,4] |
| KPC-79 | EU729727 | KPC-2 |  | ins\_269\_VYTRAPN | Yes | 4 | ≤0.03 | 34935416 | [4] |
| KPC-192\* | OR529436 | KPC-2 |  | ins\_269\_VLAVYTRAPN |  |  |  |  |  |
| KPC-129\* | ON751738 | KPC-2 | N170H | ins\_269\_KVYTRAPN |  |  |  |  |  |
| KPC-162\* | OQ579138 | KPC-2 |  | ins\_269\_KDTRAPN |  |  |  |  |  |
| KPC-108 | GQ140348 | KPC-2 |  | ins\_269\_KDDKHSEAVYTRAPN | Not reported | NA | NA | 35980232 | [5] |
| KPC-140\* | OP503888 | KPC-2 | D179N | ins\_269\_KDDKHSEAVYTRAPN |  |  |  |  |  |
| KPC-133 | OP081531 | KPC-2 | D179G | ins\_269\_KDDKHSEAVYTRAPN | Not reported | NA | NA | 27242772 | [6] |
| KPC-105\* | GQ140348 | KPC-2 | L169Q | ins\_269\_KDDKHSEAVYTRAPN |  |  |  |  |  |
| KPC-44 | MK823188 | KPC-2 |  | ins\_269\_KDDKHSEAVYTRAPN | Yes | ＞16 | 16 | 31088601 | [7] |
| KPC-148\* | JAOZYA010000028 | KPC-3 |  | ins\_269\_KDDKYSEAVYTRAPN |  |  |  |  |  |
| KPC-132\* | OP081092 | KPC-3 |  | ins\_269\_KDDKSRAP |  |  |  |  |  |
| KPC-154 | OQ096263 | KPC-3 |  | ins\_269\_KDDKYSRAP | Yes | 16 | 16 | 37877547 | [8] |
| KPC-80\* | MW444845 | KPC-2 |  | ins\_269\_KPN |  |  |  |  |  |
| KPC-193\* | OR568565 | KPC-2 |  | ins\_269\_KAN |  |  |  |  |  |
| KPC-41 | MK497255 | KPC-3 |  | ins\_269\_KPN | Yes | 128 | 0.5 | 31527032 | [9] |
| KPC-34 | KU985429 | KPC-2 |  | ins\_269\_KDDKHSEA | Not reported | NA | NA | 29855588 | [10] |
| KPC-103\* | GQ140348 | KPC-2 |  | ins\_269\_KDDKHSEAVIAA |  |  |  |  |  |
| KPC-73\* | MT833886 | KPC-2 | del\_168-169\_EL | ins\_269\_KDDKHS |  |  |  |  |  |
| KPC-163\* | OQ579139 | KPC-2 |  | ins\_269\_KDDKHS |  |  |  |  |  |
| KPC-139\* | HQ342889 | KPC-2 | D179Y | ins\_269\_KDDKHS |  |  |  |  |  |
| KPC-109\* | GQ140348 | KPC-3 |  | ins\_269\_KDDKYN |  |  |  |  |  |
| KPC-183\* | OR282800 | KPC-3 |  | ins\_269\_KDDKYS |  |  |  |  |  |
| KPC-67 | MT809697 | KPC-3 |  | ins\_269\_KDDKDD | No | ≥24 | ≥8 | 34339281 | [1] |

aVariants indicated with a star correspond to variants unpublished.

bAbbreviations: CZA, Ceftazidime-avibactam; MEM, Meropenem; NA : not applicable.

1. Carattoli, A.; Arcari, G.; Bibbolino, G.; Sacco, F.; Tomolillo, D.; Di Lella, F.M.; Trancassini, M.; Faino, L.; Venditti, M.; Antonelli, G.; et al. Evolutionary Trajectories toward Ceftazidime-Avibactam Resistance in Klebsiella Pneumoniae Clinical Isolates. Antimicrob Agents Chemother 2021, 65, e0057421, doi:10.1128/AAC.00574-21.

2. Wu, Y.; Yang, X.; Liu, C.; Zhang, Y.; Cheung, Y.C.; Wai Chi Chan, E.; Chen, S.; Zhang, R. Identification of a KPC Variant Conferring Resistance to Ceftazidime-Avibactam from ST11 Carbapenem-Resistant Klebsiella Pneumoniae Strains. Microbiol Spectr 2022, 10, e0265521, doi:10.1128/spectrum.02655-21.

3. Ding, L.; Shi, Q.; Han, R.; Yin, D.; Wu, S.; Yang, Y.; Guo, Y.; Zhu, D.; Hu, F. Comparison of Four Carbapenemase Detection Methods for blaKPC-2 Variants. Microbiol Spectr 2021, 9, e0095421, doi:10.1128/Spectrum.00954-21.

4. Shi, Q.; Han, R.; Guo, Y.; Yang, Y.; Wu, S.; Ding, L.; Zhang, R.; Yin, D.; Hu, F. Multiple Novel Ceftazidime-Avibactam-Resistant Variants of blaKPC-2-Positive Klebsiella Pneumoniae in Two Patients. Microbiol Spectr 2022, 10, e0171421, doi:10.1128/spectrum.01714-21.

5. Hobson, C.A.; Pierrat, G.; Tenaillon, O.; Bonacorsi, S.; Bercot, B.; Jaouen, E.; Jacquier, H.; Birgy, A. Klebsiella Pneumoniae Carbapenemase Variants Resistant to Ceftazidime-Avibactam: An Evolutionary Overview. Antimicrob Agents Chemother 2022, 66, e0044722, doi:10.1128/aac.00447-22.

6. Scandorieiro, S.; de Camargo, L.C.; Lancheros, C.A.C.; Yamada-Ogatta, S.F.; Nakamura, C.V.; de Oliveira, A.G.; Andrade, C.G.T.J.; Duran, N.; Nakazato, G.; Kobayashi, R.K.T. Synergistic and Additive Effect of Oregano Essential Oil and Biological Silver Nanoparticles against Multidrug-Resistant Bacterial Strains. Front Microbiol 2016, 7, 760, doi:10.3389/fmicb.2016.00760.

7. Räisänen, K.; Koivula, I.; Ilmavirta, H.; Puranen, S.; Kallonen, T.; Lyytikäinen, O.; Jalava, J. Emergence of Ceftazidime-Avibactam-Resistant Klebsiella Pneumoniae during Treatment, Finland, December 2018. Euro Surveill 2019, 24, 1900256, doi:10.2807/1560-7917.ES.2019.24.19.1900256.

8. Arcari, G.; Cecilia, F.; Oliva, A.; Polani, R.; Raponi, G.; Sacco, F.; De Francesco, A.; Pugliese, F.; Carattoli, A. Genotypic Evolution of Klebsiella Pneumoniae Sequence Type 512 during Ceftazidime/Avibactam, Meropenem/Vaborbactam, and Cefiderocol Treatment, Italy. Emerg Infect Dis 2023, 29, 2266–2274, doi:10.3201/eid2911.230921.

9. Mueller, L.; Masseron, A.; Prod’Hom, G.; Galperine, T.; Greub, G.; Poirel, L.; Nordmann, P. Phenotypic, Biochemical, and Genetic Analysis of KPC-41, a KPC-3 Variant Conferring Resistance to Ceftazidime-Avibactam and Exhibiting Reduced Carbapenemase Activity. Antimicrobial Agents and Chemotherapy 2019, 63, 10.1128/aac.01111-19, doi:10.1128/aac.01111-19.

10. Chiu, S.-K.; Ma, L.; Chan, M.-C.; Lin, Y.-T.; Fung, C.-P.; Wu, T.-L.; Chuang, Y.-C.; Lu, P.-L.; Wang, J.-T.; Lin, J.-C.; et al. Carbapenem Nonsusceptible Klebsiella Pneumoniae in Taiwan: Dissemination and Increasing Resistance of Carbapenemase Producers During 2012-2015. Sci Rep 2018, 8, 8468, doi:10.1038/s41598-018-26691-z.