Phenotypic and Genotypic Characterization of Enterobacteriaceae Producing Oxacillinase-48–Like Carbapenemases, United States

Technical Appendix

Additional Materials and Methods

To assess clonality of plasmid sequences in our dataset, we mapped Illumina (San Diego, CA) reads from each β-lactamase oxacillinase-48–like (blaOXA-48-like) isolate to the appropriate internal reference isolate sequenced by using Pacific Biosciences (Menlo Park, CA, USA) long-read technology by using the lyve-SET V1.0 pipeline (https://github.com/lskatz/lyve-SET). Single-nucleotide polymorphisms (SNPs) with 100% consensus and ≥5× sequencing depth were reported (1). We measured and visualized the number of SNPs in each pairwise comparison of a mapped isolate and the reference plasmid (Pacific Biosciences), as well as the total number of read-covered nucleotides on each reference plasmid assembly (depth of coverage ≥5×), in a scatter plot by using R 3.2.3 statistical software (https://www.r-project.org/).

All Illumina contigs that harbored blaOXA-48-like genes were extracted from each genome assembly and aligned against the GenBank database by means of BLASTN (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=tblastn&PAGE_TYPE=BlastSearch) to estimate the genomic location of the blaOXA-48–like gene (either plasmid or chromosome) and were also screened for plasmid replicon sequences by using the PlasmidFinder database (http://www.genomicepidemiology.org/).

A phylogenetic tree was inferred from whole-genome SNP data of 7 sequence type 34 Klebsiella pneumoniae isolates by means of the Lyve-Set1.1.4f pipeline (1). First, phage genes present in the genome sequence (Pacific Biosciences) of isolate 1 (K. pneumoniae sequence 34 isolate) were identified by screening against the PHAST database (2), and trimmed Illumina reads were subsequently mapped to unmasked regions of the reference by using SMALT (3).
SNPs with \( \geq 95\% \) consensus and \( \geq 20\times \) sequencing depth were reported as high-quality SNPs. To deal with possible regions of homologous recombination, leading to high-density SNP regions, we accepted 1 SNP/5 bp, a flanking distance close to the average recombination cassette length (4). A maximum-likelihood phylogeny was inferred from high-quality SNPs by using RAxML version 8 (5) and a generalized time reversible substitution model and a gamma model of rate heterogeneity.

**Additional Results**

The number of core plasmid SNPs between isolate 11 (pColKP3_DHQP1300920) (sequenced by using Illumina and Pacific Biosciences technology) and other isolates with *bla*\_OXA-232 (sequenced by using Illumina technology) ranged from 0 to 1 and covered 5,400–6,100 bp (Technical Appendix Figure 1). Among the isolates with *bla*\_OXA-48, the number of core SNPs between the plasmid sequence from reference isolate 23 (Pacific Biosciences) (pIncL\_M\_DHQP\_1400954) and those from other OXA-48 isolates (sequenced by using Illumina technology) ranged from 371 to 430 and covered 56,000 and 62,000 bp (Technical Appendix Figure 2).

Alignments of Illumina contigs harboring *bla*\_OXA-48-like run against the GenBank database showed that all contigs mapped to plasmid sequences. Alignments of Illumina contigs with *bla*\_OXA-181 run against the GenBank database estimated 9 isolates to have a chromosomal location and isolates 8, 13, 20, and 24 to have a plasmid location. In 3 instances (isolates 8, 13, and 24), *bla*\_OXA-181 was found on the same contig as a plasmid replicon gene (2 ColKP3 replicons and 1 IncHI1B replicon), further suggesting that *bla*\_OXA-181 was located on a plasmid in those isolates.

**References**


![Technical Appendix Figure 1](image)

**Technical Appendix Figure 1.** Genetic diversity among ColKP3 plasmids that harbor β-lactamase oxacillinase-232. SNPs were identified by mapping trimmed Illumina (San Diego, CA, USA) sequencing reads to a fully closed PacBio ColKP3 plasmid assembly (isolate 11). The y-axis shows the amount of Pacific Biosciences (Menlo Park, CA, USA) reference nucleotides covered by Illumina reads of each sample (minimum of 5× coverage), and the x-axis shows the quantity of SNPs observed between the Pacific Biosciences reference and each isolate. Boxplot indicates 25th–75th interquartile ranges and relatedness among these type of oxacillinase-232–carrying plasmids in terms of shared nucleotide positions between isolates and the reference. Horizontal line in box indicates median. Outermost line (error bar) indicates 1.5× interquartile range. Only 2 isolates are considered outliers and thus more distantly related. SNP count variation ranges from 0 to 1 and indicates relatedness between isolates and the reference. SNP, single-nucleotide polymorphism.
Technical Appendix Figure 2. Genetic diversity among IncL/M plasmids that harbor β-lactamase oxacillinase-48. SNPs were identified by mapping trimmed Illumina sequencing reads to a fully closed (Pacific Biosciences, Menlo Park, CA, USA) IncL/M plasmid assembly (isolate 23). The y-axis shows the amount of Pacific Biosciences reference nucleotides covered by Illumina reads of each sample (minimum of 5× coverage), and the x-axis shows the quantity of SNPs observed between the Pacific Biosciences reference and each isolate. Boxplots indicate 25th–75th interquartile ranges and show that 100% coverage and 0 SNPs (indicating 100% sequence similarity) are considered features of an outlier and therefore rare in this dataset. Horizontal lines indicate medians. Outermost lines (error bars) indicate 1.5× interquartile ranges. Most IncL/M plasmids are distantly related to the Pacific Biosciences plasmid reference, indicating substantial heterogeneity of plasmids within this dataset. SNP, single-nucleotide polymorphism.