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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 ($bla_{\text{OXA-48–}}$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 $bla_{\text{OXA-48–}}$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=BlastSearchto) estimate the genomic location of the $bla_{\text{OXA-48–}}$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 ≥95% consensus and ≥20× 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 $bla_{\text{OXA-232}}$ (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 $bla_{\text{OXA-48}}$, 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 $bla_{\text{OXA-48}}$–like run against the GenBank database showed that all contigs mapped to plasmid sequences. Alignments of Illumina contigs with $bla_{\text{OXA-181}}$ 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), $bla_{\text{OXA-181}}$ was found on the same contig as a plasmid replicon gene (2 ColKP3 replicons and 1 IncHI1B replicon), further suggesting that $bla_{\text{OXA-181}}$ was located on a plasmid in those isolates.

References

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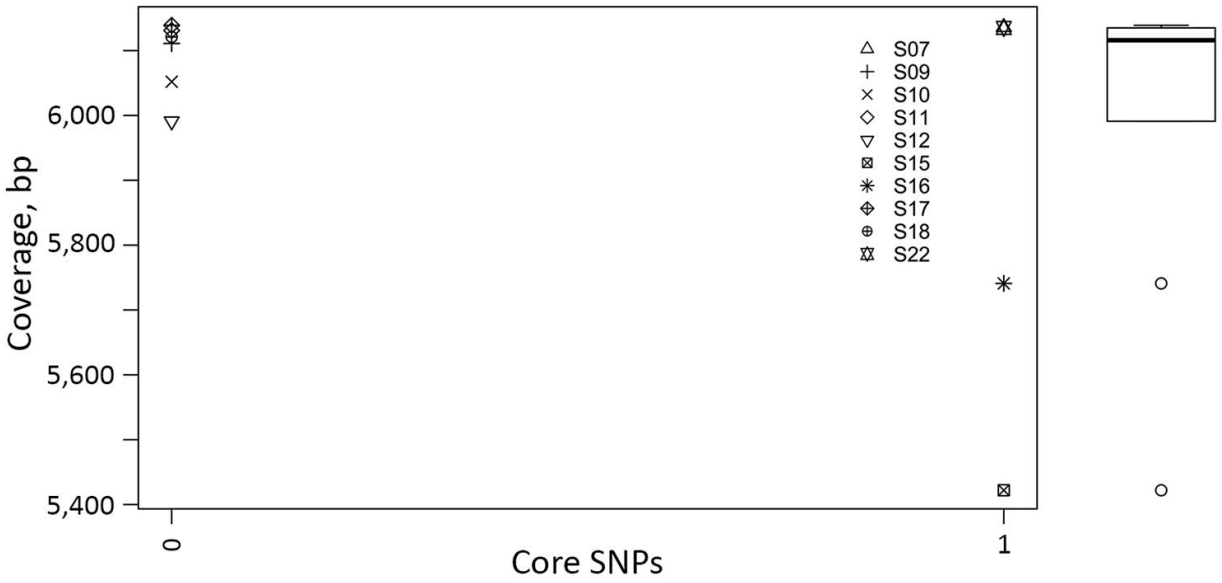
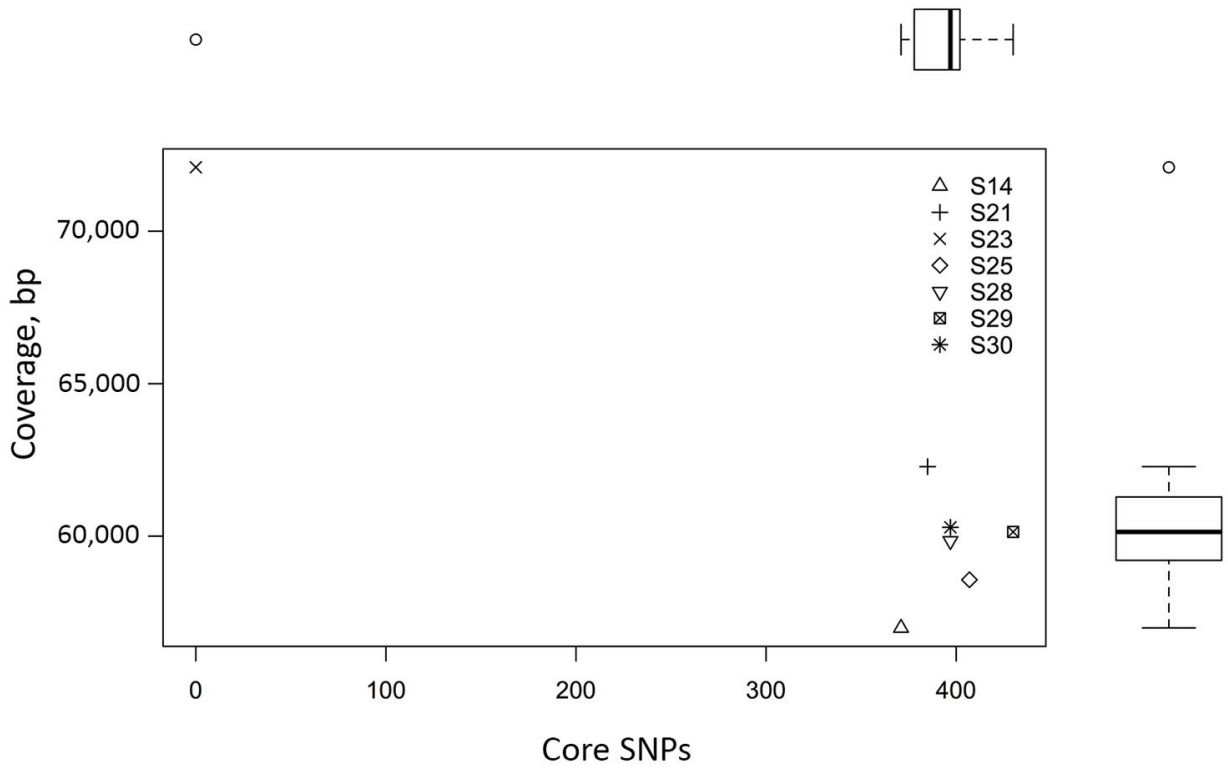


Technical Appendix Figure 1. Genetic diversity among ColKP3 plasmids that harbor β-lactamase oxacillinase-232. SNPs were identified by mapping trimmed Illumina (San Diego, CA, USA) sequencing reads to a fully closed PacBio ColKP3 plasmid assembly (isolate 11). The y-axis shows the amount of Pacific Biosciences (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.