Machine learning models to identify patient and microbial genetic factors associated with carbapenem-resistant *Klebsiella pneumoniae* infection

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Abstract

Background

Among patients colonized with carbapenem-resistant *Klebsiella pneumoniae* (CRKP), only a subset develop clinical infection. While patient characteristics may influence risk for infection, it remains unclear if the genetic background of CRKP strains contributes to this risk. We applied machine learning to quantify the capacity of patient characteristics and microbial genotypes to discriminate infection and colonization, and identified patient and microbial features associated with infection across multiple healthcare facilities.

Methods

Machine learning models were built using whole-genome sequences and clinical metadata from 331 patients colonized or infected with CRKP across 21 long-term acute care hospitals. To quantify variation in performance, we built models using 100 different train/test splits of the entire dataset, and urinary and respiratory site-specific subsets, and evaluated predictive performance on each test split using the area under the receiver operating characteristics curve (AUROC). Patient and microbial features predictive of infection were identified as those consistently important for predicting infection based on average change in AUROC when included in the model.

Findings

We found that patient and genomic features were only weakly predictive of clinical CRKP infection vs. colonization (AUROC IQRs: patient=0.59-0.68, genomic=0.55-0.61, combined=0.62-0.68), and that one feature set did not consistently outperform the other (genomic vs. patient p=0.4). Comparable model performances were observed for anatomic site-specific models (combined AUROC IQRs: respiratory=0.61-0.71, urinary=0.54-0.64). Strong genomic predictors of infection included the presence of the ICEKp10 mobile genetic element carrying an iron acquisition system (yersiniabactin) and a toxin (colibactin), along with disruption of an O-antigen biosynthetic gene in a sub-lineage of the epidemic ST258 clone. Teasing apart sequential evolutionary steps in the context of clinical metadata indicated that
altered O-antigen biosynthesis increased association with the respiratory tract, and subsequent acquisition of ICEKp10 was associated with increased virulence.

**Interpretation**

Our results support the need for rigorous machine learning frameworks to gain realistic estimates of the performance of clinical models of infection. Moreover, integrating microbial genomic and clinical data using such a framework can help tease apart the contribution of microbial genetic variation to clinical outcomes.

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**Research in context**

**Evidence before this study**

We searched PubMed for "crkp" OR "carbapenem resistant klebsiella pneumoniae" AND "infection" AND "machine learning" for papers published up to April 14, 2020 and found no results. Substituting “machine learning” with "bacterial genome-wide association studies" produced one relevant paper investigating pathogenicity-associated loci in *K. pneumoniae* clinical isolates. When we searched for "infection" AND "machine learning" AND "genom*" AND "clinical", there was one relevant result - a study that used clinical and bacterial genomic features in a machine learning model to identify clonal differences related to *Staphylococcus aureus* infection outcome.

**Added value of this study**

To our knowledge, this is the first study to integrate clinical and genomic data to study anatomic site-specific colonization and infection across multiple healthcare facilities. Using this method, we identified clinical features associated with CRKP infection, as well as a sub-lineage of CRKP with potentially altered niche-specific adaptation and virulence. This method could be used for other organisms and other
clinical outcomes to evaluate performance of predictive models and identify features that are consistently
associated with clinical outcomes of interest across facilities or geographic regions.

**Implications of all the available evidence**

Few studies have combined patient and microbial genomic data to study important clinical outcomes. However, those that have done this, including ours, have identified clinical and/or genomic features associated with the outcome of interest that provide a foundation for future epidemiological, clinical, and biological studies to better understand bacterial infections and clinical outcomes.

**Introduction**

Infections due to multidrug resistant organisms (MDROs) lead to hundreds of thousands of deaths worldwide each year.\(^1\) Carbapenem-resistant Enterobacterales (CRE) is a critical-priority antibiotic resistance threat that has emerged over the past several decades, spread across the globe, and accumulated resistance to last-line antibiotic agents.\(^2,3\) In the United States (US), CRE infections are primarily caused by the sequence type (ST) 258 strain of carbapenem resistant *Klebsiella pneumoniae* (CRKP), which has become endemic in regional healthcare networks.\(^3-7\) In this background of regional endemicity the risk of patient exposure to CRKP is high, as evidenced by alarmingly high rates of colonization, especially in long-term care settings.\(^7,8\) However, even among critically ill patients residing in long-term care facilities, not all colonized patients develop clinical infections that require antibiotic treatment.\(^9\) Currently, our understanding of the factors that influence whether a colonized patient develops an infection is incomplete.

In addition to clinical characteristics of patients, the genetic background of the colonizing strain may also influence the risk of infection, as there is extensive intra-species variation in antibiotic resistance and virulence determinants harbored by *K. pneumoniae*.\(^3\) To date, most studies of virulence determinants have been carried out in model systems,\(^10\) or examined in human populations without considering patient characteristics or clinical context.\(^11\) One recent study investigated virulence determinants in *K.*
pneumoniae clinical isolates while controlling for patient characteristics. However, this was a single-site study with a focus on carbapenem-susceptible K. pneumoniae, thereby not addressing the impact of genomic variation in antibiotic-resistant lineages that circulate in global healthcare systems.

Here, we sought to understand the importance of both patient factors and CRKP genetic background in determining whether a patient is infected (vs. colonized) with CRKP, and identify a set of patient and microbial features that are consistent predictors of CRKP infection across long-term care facilities. To accomplish this, we compared patients with CRKP colonization and infection based upon both clinical characteristics and the genomes of their colonizing or infecting strains. To improve the generalizability of our findings, we employed a rigorous machine learning framework and included patients from 21 long-term acute care hospitals (LTACHs) across the US.

**Methods**

**Clinical and genomic data**

We used whole-genome sequences of clinical (non-surveillance) CRKP isolates and associated patient metadata from a prospective observational study performed in 21 LTACHs from across the US over the course of a year (BioProject accession no. PRJNA415194). We included only the first clinical bloodstream, respiratory, or urinary isolate from each patient (n=355; Figure S1A), and subset to only ST258 isolates for the majority of analyses (n=331; Table S1; see supplementary material for reasoning).

Details about the analysis pipeline, genomic data curation, and phylogenetic tree reconstruction are provided in the supplementary material.

**Outcome definition**

Our outcome of interest was colonization vs. clinical infection (Figure S1B). Based on established Centers for Disease Prevention and Control’s National Healthcare Safety Network (CDC’s NHSN) surveillance definitions, we considered all bloodstream isolates as representative of infection, and used modified definitions to classify urinary and respiratory cultures as representative of infection versus
Any isolate that did not meet the criteria for infection was classified as colonization. **Feature sets**

We studied the association between five different feature sets and infection/colonization in CRKP ST258 (Figure S1C), described below. See supplementary methods for details on feature set creation and processing. Counts below are for confident features from the entire dataset prior to processing.

**Patient:** Clinical features described in Han et al.\(^{13}\) (n=50; **Table S3**).

**Uncurated genomic:** single nucleotide variants, indels, insertion sequence elements, and pangenome genes (n=2447).

**Uncurated grouped genomic:** Gene-level variant presence/absence and pangenome genes (n=3159).

**Curated genomic:** Features identified by Kleborate,\(^{15}\) a tool designed to identify the presence of various genes and mutations known to be associated with either CRKP virulence or antibiotic resistance (n=91).

**Patient & curated genomic:** Patient features and curated genomic features (n=141).

**Machine learning & model selection**

We aimed to classify clinical infection (vs. colonization) using each of the different feature sets (see above); we built classifiers using the first clinical isolate from each patient for all isolates, only respiratory isolates, and only urinary isolates. We performed L2 regularized logistic regression using a modified version of the machine learning pipeline presented in Topçuoğlu et al.\(^{27}\) using caret version 6.0-85\(^{28}\) in R version 3.6.2\(^{29}\) (Figure S1D1). We randomly split the data into 100 unique ~80/20 train/test splits, keeping all isolates from each LTACH grouped in either the training set or the held-out test set to control for facility-level differences among the isolates (e.g., background of circulating strains within each facility, patient population, and clinician test ordering frequency). For valid comparison, the train/test splits were identical across models generated with different feature sets. Hyperparameters were selected via cross-validation on the training set to maximize the average AUROC across cross-validation folds. See supplementary methods for more details.
Model performance

We measured model performance using the median test area under the receiver operating characteristic curve (AUROC) and area under the precision recall curve (AUPRC), as well as the interquartile range, across all 100 train/test splits (Figure S1D2).

Features consistently associated with colonization or infection

To determine the importance of each feature in predicting colonization vs. infection, we measured how much each feature influenced model performance by calculating a permutation importance (Figure S1D3). For each combination of feature and data split, we randomly permuted the feature and calculated the ‘permuted test AUROC’ using the model generated with the training data. Features with a correlation of 1 were permuted together. We performed this permutation test 100 times for each feature/data split pair, and obtained a mean permutation importance for each data split. A mean permutation importance above zero indicates that that feature improved model performance for that data split. We highlight features where the mean test AUROC was above zero in at least 75% of the data splits. In this way, the permutation importance method allows us to take into account the variation we observe across the 100 models, which is not possible with standard parametric statistical tests or odds ratios.

Data analysis & visualization

See supplementary material for details on data analysis and visualization in R version 3.6.2. All code and data that is not protected health information is on GitHub (https://github.com/Snitkin-Lab-Umich/ml-crkp-infection-manuscript).

Role of the funding source

The funding source had no role in study design; data collection, analysis, and interpretation; or report writing. All authors had full access to all data in the study and final responsibility for the decision to submit for publication.
Results

Of the 355 clinical CRKP isolates from 21 LTACHs across the US,\textsuperscript{13} we classified 149 (42\%) of the isolates as representing infection based on modified NHSN criteria (Figure S2, Tables S1-3). Stratified by anatomic site, we classified 29/29 (100\%) blood isolates as infection, 69/196 (35\%) respiratory isolates as infection, and 51/130 (39\%) urinary isolates as infection (Table S3). More than 90\% of patient isolates were from the epidemic CRKP lineage ST258 (Tables S1). Patients harboring different sequence types of CRKP showed no significant differences in infection/colonization status or anatomic site of isolation, and no substantive differences in clinical characteristics (see supplementary material). Thus, we decided to limit our analysis to ST258 to improve our ability to discern whether genetic variation in this dominant strain is associated with infection.

The CRKP epidemic lineage ST258 shows evidence of sub-lineage variation in virulence and anatomic site of isolation

We next evaluated if there exist sub-lineages of ST258 with altered virulence properties by looking for clustering of isolates by infection on the whole-genome phylogeny (Figure 1; see supplementary methods).\textsuperscript{36} Infection status was non-randomly distributed on the phylogeny (p=0.002), supporting our hypothesis that the genetic background of CRKP influences infection. We performed a similar clustering analysis to look at potential niche-specific adaptation to certain anatomic sites (Figure 1), and found that respiratory (p=0.001) and urinary (p=0.013) isolates cluster on the phylogeny, but blood isolates do not (p=0.21). This analysis indicates that, in addition to patient features, intra-strain variation in virulence and adaptation to the urinary and respiratory tract might influence whether patients develop an infection.

Both patient and CRKP genetic characteristics are weakly predictive of infection, with relative performance being highly facility-dependent

We next performed machine learning to quantify the ability of patient and microbial genetic characteristics to predict CRKP infection (Figure S1). To prevent over- or under-fitting and control for facility-level biases, we generated 100 train/test data splits, wherein a given LTACH was only included either in the train or test set. Each LTACH occurred a median of 24 times (range 13-32) in the test data.
split. In this way, we were able to identify patient and CRKP strain characteristics consistently associated with infection or colonization across data splits, and thus across patient populations in different healthcare facilities.

First, we sought to understand if patient and genomic features were individually predictive of CRKP infection. To this end, we independently evaluated patient characteristics as well as three different genomic feature sets for their ability to classify colonization and infection (see methods). Across the 100 different train/test splits, we observed that the average predictive performance was weak, with each of the genomic and patient feature sets predictive of infection to a similar degree (all 1st quartile AUROCs > 0.5; median range=0.55-0.68; Figure 2A; AUPRC: Figure S3A). Across the 100 different data splits, no one feature set was consistently the most predictive (e.g. Figures 2B, 2C; all comparisons p > 0.30, see supplementary methods for p-value calculation). Furthermore, for each feature set AUROCs were distributed such that the test AUROC ranged from below 0.5 to over 0.7, depending on how the data were split (i.e., which facilities appear in the train/test sets). This variation in model performance across different train/test sets suggests that the association of CRKP strain and patient characteristics with infection or colonization varies across facilities.

Integration of patient and CRKP strain features does not improve discriminative performance of overall or anatomic site-specific models

To determine if the predictive power of patient and genomic features is additive, and if combining these disparate feature sets improved validation on held-out facilities, we built models including both patient and curated genomic features. The discriminative performance of the models based on the combined feature set was not significantly greater than that of the individual feature sets (Figure 2A, p ≥ 0.20).

Thus, despite variation in the predictive capacity of genomic and patient features across facilities (Figure 2C), combining the two sets did not improve overall performance. Focusing on anatomic site-specific models revealed similar trends, where classification performances were similar for respiratory and urinary specific models, and the relative predictive capacity of patient and CRKP strain features varied across facility subsets (Figure S4; AUPRC: Figure S3B).
Some patient and genomic features consistently discriminate colonization and infection

After evaluating the predictive capacity of models, we next sought to identify patient and CRKP strain characteristics that are most associated with CRKP infection or colonization. To this end, we identified those patient and genomic features that consistently improved model performance across the 100 different data splits (see methods). Evaluating the importance of features in this way provides insight into those characteristics that generalize across different facility subsets. This approach was taken for both overall and anatomic site-specific models to identify features predictive of different anatomic sites of infection (Figure 3, Figures S5-7).

Several patient features were consistently associated with infection in the overall analysis, including presence of a gastrostomy tube, presence of a central venous catheter, acute kidney injury, and severe chronic kidney disease (Figure 3), all markers of critically ill patients. Only a small number of genomic features were consistently associated with infection (Figure 3). No known virulence factors were positively associated with colonization; all of the genomic features positively associated with colonization are antibiotic resistance elements. Conversely, all but one of the genomic features positively associated with infection (3/4) are related to virulence. The ICEKp10 element is positively associated with infection and carries colibactin and two different types of yersiniabactin (Figure S8). Additionally, insertion sequence-mediated disruption of the O-antigen biosynthetic gene kfoC (see supplementary methods and Figure S9 for insertion sequence identification) was associated with respiratory infection.

Colibactin is a toxin, and yersiniabactin is an iron scavenging system that has been identified in previous animal and human studies as being associated with virulence. The O-antigen of lipopolysaccharide (LPS) is a known antigenic marker, although association with a specific anatomic site has not been noted.

A sub-lineage of ST258 clade II appears to have sequentially evolved enhanced adaptation for the respiratory tract and increased virulence

We noted that kfoC disruption is largely confined to a sub-lineage of ST258 (Figures 4, S10, S11).

Consistent with this feature being associated with respiratory infection, the disrupted kfoC lineage is
enriched in respiratory isolates (82/118, 69% of isolates in the disrupted kfoC lineage are respiratory isolates vs. 101/213, 47% in all other isolates; Fisher’s exact p=0.0001), suggesting that this lineage is associated with increased capacity for respiratory colonization. Furthermore, a subset of isolates in the disrupted kfoC sub-lineage contain the ICEKp10 element. Examination of these genetic events in the context of the whole-genome phylogeny revealed that disruption of kfoC occurred first, followed by at least two different acquisitions of ICEKp10 (Figures 4, S10). Within the disrupted kfoC lineage, isolates with ICEKp10 are enriched in infection (31/55, 56% of isolates with ICEKp10 are infection isolates vs. 16/63, 25% of isolates without ICEKp10, Fisher’s exact p = 0.00065), supporting an increase in virulence after acquisition of ICEKp10. It is important to note that the observed clinical associations with ICEKp10 and kfoC disruption do not demonstrate causality, as we cannot rule out the role of correlated genetic variation.

Discussion

There have been numerous studies aimed at identifying risk factors for healthcare-associated infections caused by prominent antibiotic-resistance threats. For the most part, these studies have found the dominant risk factors to be linked to the magnitude of exposure (e.g. length of stay or colonization pressure), use of antibiotics, and overall comorbidity. Here we found similar results, where length of stay and having certain comorbidities were positively associated with infection. What remains unclear is whether, in the critically ill populations heavily exposed to antibiotics that are at greatest risk, if genetic variation in circulating resistant lineages influences patient infection status. Here, we addressed this question by focusing on CRKP infection in a cohort of patients from 21 LTACHs across the US. To gain a realistic assessment of the predictive capacities of patient and CRKP genetic features, we employed a machine learning framework using multiple facility-level train/test splits. Overall, we found that, while neither patient nor CRKP genetic features have high predictive accuracy on held-out test data, both feature sets were independently associated with infection, with one or the other being more predictive on different facility subsets. Moreover, the integration of clinical and genomic data led to the discovery of an
emergent sub-lineage of the epidemic ST258 clone that may have increased adaptation for the respiratory tract, and is more strongly associated with infection.

One strength of our machine learning approach is that we were able to measure the variation in discriminative performance across 100 train/test iterations that differed in which facilities were included in train and test sets. We found that performance varied greatly depending on how facilities were allocated to train and test sets, highlighting how smaller studies could overestimate or underestimate the discriminative ability of both their model and individual features. One potential explanation for variation in model performance is that there is facility-level heterogeneity depending on their characteristics (e.g. size, geography, etc.), in which case building sub-models for relevant facility subsets may improve performance. Another possible explanation for variation in model performance may be that the critically-ill nature of LTACH patients is such that most patients are actually highly susceptible to infection (i.e. many patients colonized with CRKP may ultimately develop an infection). However, it's noteworthy that despite these potential challenges in creating generalizable models, our analysis did yield predictors of infection and colonization consistent across test sets, and thus across LTACHs.

We built classifiers including all genomic features as well as a curated subset of features, and found that both are similarly weakly predictive of infection. However, while the uncurated feature set presented challenges with downstream interpretation, our analyses on the curated genomic features facilitated novel insights into potential evolutionary trajectories of anatomic site-specific adaptation and virulence. For example, we observed that disruption of the O-antigen biosynthetic gene, kfoC, is associated with isolation from the respiratory tract. While we cannot determine from our machine learning analysis if disruption of kfoC is directly causal, the biological plausibility of an altered O-antigen structure mediating evasion of innate immunity and/or other beneficial interactions with the host makes this a strong candidate for followup experiments. Supporting this hypothesis, a previous study found that absence of O-antigen is associated with decreased virulence, but not decreased intrapulmonary proliferation, in a murine model. In addition, we noted that a number of antibiotic resistance determinants were associated with colonization. We hypothesize that this observation could be a consequence of longer duration of
Finally, we also saw evidence that, after acquiring yersiniabactin and colibactin on the ICEKp10 element, the disrupted $kfoC$ subclade became more strongly associated with infection, supporting the idea that circulating ST258 sub-lineages can evolve to become both hypervirulent and multi-drug resistant.\(^{18,43,45}\)

Our study has several important limitations. Specifically, CRKP colonization vs. infection for non-bloodstream isolates may be difficult to discriminate based on surveillance criteria and the clinical data that were available. However, we based our definitions on established CDC criteria with modifications used previously.\(^7\) Encouragingly, we were still able to identify consistent predictors of infection, even with potential misclassifications. A second limitation is that we were limited in the patient data included in our model. It is likely that important differences in underlying patient conditions were not captured by the coarse clinical variables we included, and we also did not account for differences in genetic variation in the host.\(^{46}\) Other limitations include that our study was restricted to LTACH patients, and had non-random geographic sampling. While LTACHs have unique structural features, based on prior studies, we expect that the types of patient risk factors considered are likely to generalize to other patient populations. Moreover, our restriction to LTACHs in endemic geographic regions has the benefit of focusing on populations at disproportionate risk for CRKP infection.\(^8\) Finally, while the employed machine learning approach allowed for meaningful assessment of discriminative power using a large number of features, by nature it does not yield estimates of attributable risk. However, features identified as consistently associated with colonization or infection on held-out test data can be evaluated by epidemiologists, clinicians, and biologists to identify potential targets for follow-up epidemiologic or laboratory studies.

**Conclusion**

We employed a machine learning approach to quantify our ability to discriminate between CRKP colonization and infection using patient and microbial genomic features. This approach highlighted the high degree of variation in predictive accuracy across different facility subsets. Furthermore, despite modest predictive power, we identified several genomic features consistently associated with infection,
indicating that variation in circulating CRKP strains contributes to infection, even in the context of the critically-ill patient populations residing in LTACHs. Future work should aim to corroborate our findings with larger cohorts, and follow up on strong associations to determine whether they are indeed risk factors for infection. This could ultimately help identify patients at high risk for infection and devise targeted strategies for infection prevention.

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Author contributions

ESS, JH, EL, and ZL conceptualized the study and acquired funding to support the project. JH and ZL performed data curation. ZL performed formal analysis, investigation, and visualization. ESS, JW, and ZL developed methodology. ESS supervised the project. ZL and ESS wrote the original draft. All authors reviewed and edited the manuscript.

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Conflicts of interest

JHH was employed at the University of Pennsylvania during the conduct of this study. She is currently an employee of, and holds shares in, the GSK group of companies.

References

1. Organization WH. Antimicrobial Resistance: Global Report on Surveillance. Geneva: World Health Organization; 2014. 232 p.

2. Munoz-Price LS, Poirel L, Bonomo RA, Schwaber MJ, Daikos GL, Cormican M, et al. Clinical epidemiology of the global expansion of Klebsiella pneumoniae carbapenemases. Lancet Infect Dis. 2013 Sep;13(9):785–96.

3. Wyres KL, Lam MMC, Holt KE. Population genomics of Klebsiella pneumoniae. Nature Reviews Microbiology. 2020 Feb 13;1–16.

4. Ansari U, Lawsin A, Campbell D, Albrecht V, McAllister G, Bulens S, et al. Molecular Characterization of Carbapenem-Resistant Enterobacteriaceae in the USA, 2011–2015. Open Forum Infect Dis. 2017 Oct 1;4(suppl_1):S179–S179.

5. Logan LK, Weinstein RA. The Epidemiology of Carbapenem-Resistant Enterobacteriaceae: The Impact and Evolution of a Global Menace. J Infect Dis. 2017 Feb 15;215(Suppl 1):S28–36.

6. Lee C-R, Lee JH, Park KS, Kim YB, Jeong BC, Lee SH. Global Dissemination of Carbapenemase-Producing Klebsiella pneumoniae: Epidemiology, Genetic Context, Treatment Options, and Detection Methods. Front Microbiol [Internet]. 2016 [cited 2018 Feb 10];7. Available from: https://www.frontiersin.org/articles/10.3389/fmicb.2016.00895/full

7. Han JH, Goldstein EJC, Wise J, Bilker WB, Tolomeo P, Lautenbach E. Epidemiology of Carbapenem-Resistant Klebsiella pneumoniae in a Network of Long-Term Acute Care Hospitals.
Clin Infect Dis. 2017 Apr 1;64(7):839–44.

8. Lin MY, Lyles-Banks RD, Lolans K, Hines DW, Spear JB, Petrak R, et al. The Importance of Long-term Acute Care Hospitals in the Regional Epidemiology of Klebsiella pneumoniae Carbapenemase–Producing Enterobacteriaceae. Clin Infect Dis. 2013 Nov 1;57(9):1246–52.

9. Lee BY, Bartsch SM, Wong KF, Kim DS, Cao C, Mueller LE, et al. Tracking the spread of carbapenem-resistant Enterobacteriaceae (CRE) through clinical cultures alone underestimates the spread of CRE even more than anticipated. Infection Control & Hospital Epidemiology. 2019 Jun;40(6):731–4.

10. Bachman MA, Oyler JE, Burns SH, Caza M, Lépine F, Dozois CM, et al. Klebsiella pneumoniae Yersiniabactin Promotes Respiratory Tract Infection through Evasion of Lipocalin 2. Infection and Immunity. 2011 Aug 1;79(8):3309–16.

11. Holt KE, Wertheim H, Zadoks RN, Baker S, Whitehouse CA, Dance D, et al. Genomic analysis of diversity, population structure, virulence, and antimicrobial resistance in Klebsiella pneumoniae, an urgent threat to public health. PNAS. 2015 Jul 7;112(27):E3574–81.

12. Martin RM, Cao J, Wu W, Zhao L, Manthei DM, Pirani A, et al. Identification of Pathogenicity-Associated Loci in Klebsiella pneumoniae from Hospitalized Patients. mSystems [Internet]. 2018 Jun 26 [cited 2020 Apr 16];3(3). Available from: https://msystems.asm.org/content/3/3/e00015-18

13. Han JH, Lapp Z, Bushman F, Lautenbach E, Goldstein EJC, Mattei L, et al. Whole-Genome Sequencing To Identify Drivers of Carbapenem-Resistant Klebsiella pneumoniae Transmission within and between Regional Long-Term Acute-Care Hospitals. Antimicrobial Agents and Chemotherapy [Internet]. 2019 Nov 1 [cited 2019 Dec 18];63(11). Available from: https://aac.asm.org/content/63/11/e01622-19

14. Köster J, Rahmann S. Snakemake—a scalable bioinformatics workflow engine. Bioinformatics. 2012 Oct 1;28(19):2520–2.

15. Holt K. katholt/Kleborate [Internet]. 2020 [cited 2020 Apr 15]. Available from: https://github.com/katholt/Kleborate
16. Wyres KL, Wick RR, Gorrie C, Jenney A, Follador R, Thomson NR, et al. Identification of Klebsiella capsule synthesis loci from whole genome data. Microbial Genomics, 2016;2(12):e000102.

17. Wick RR, Heinz E, Holt KE, Wyres KL. Kaptive Web: User-Friendly Capsule and Lipopolysaccharide Serotype Prediction for Klebsiella Genomes. Journal of Clinical Microbiology [Internet]. 2018 Jun 1 [cited 2020 Feb 20];56(6). Available from: https://jcm.asm.org/content/56/6/e00197-18

18. Lam MMC, Wick RR, Wyres KL, Gorrie CL, Judd LM, Jenney AWJ, et al. Genetic diversity, mobilisation and spread of the yersiniabactin-encoding mobile element ICEKp in Klebsiella pneumoniae populations. Microbial Genomics, 2018;4(9):e000196.

19. Cingolani P, Platts A, Wang LL, Coon M, Nguyen T, Wang L, et al. A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff. Fly. 2012 Apr 1;6(2):80–92.

20. Treepong P, Guyeux C, Meunier A, Couchoud C, Hocquet D, Valot B. panISa: ab initio detection of insertion sequences in bacterial genomes from short read sequence data. Bioinformatics. 2018 Nov 15;34(22):3795–800.

21. Page AJ, Cummins CA, Hunt M, Wong VK, Reuter S, Holden MTG, et al. Roary: rapid large-scale prokaryote pan genome analysis. Bioinformatics. 2015 Nov 15;31(22):3691–3.

22. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, et al. The Sequence Alignment/Map format and SAMtools. Bioinformatics. 2009 Aug 15;25(16):2078–9.

23. Croucher NJ, Page AJ, Connor TR, Delaney AJ, Keane JA, Bentley SD, et al. Rapid phylogenetic analysis of large samples of recombinant bacterial whole genome sequences using Gubbins. Nucleic Acids Res. 2015 Feb 18;43(3):e15–e15.

24. Nguyen L-T, Schmidt HA, von Haeseler A, Minh BQ. IQ-TREE: A Fast and Effective Stochastic Algorithm for Estimating Maximum-Likelihood Phylogenies. Mol Biol Evol. 2015 Jan;32(1):268–74.

25. Minh BQ, Nguyen MAT, von Haeseler A. Ultrafast Approximation for Phylogenetic Bootstrap. Mol
26. 2020 NHSN Patient Safety Component Manual. 2020;434.

27. Topçuoğlu BD, Lesniak NA, Ruffin MT, Wiens J, Schloss PD. A Framework for Effective Application of Machine Learning to Microbiome-Based Classification Problems. mBio [Internet]. 2020 Jun 30 [cited 2020 Jun 23];11(3). Available from: https://mbio.asm.org/content/11/3/e00434-20

28. Kuhn M. Building Predictive Models in R Using the caret Package. Journal of Statistical Software. 2008 Nov 10;28(1):1-26.

29. R: The R Project for Statistical Computing [Internet]. [cited 2020 Apr 15]. Available from: https://www.r-project.org/

30. Wickham H, Averick M, Bryan J, Chang W, McGowan L, François R, et al. Welcome to the Tidyverse. Journal of Open Source Software. 2019 Nov 21;4(43):1686.

31. Wilke CO. cowplot: Streamlined Plot Theme and Plot Annotations for “ggplot2” [Internet]. 2019 [cited 2020 Apr 15]. Available from: https://CRAN.R-project.org/package=cowplot

32. Yu G, Smith DK, Zhu H, Guan Y, Lam TT-Y. ggtree: an r package for visualization and annotation of phylogenetic trees with their covariates and other associated data. Methods in Ecology and Evolution. 2017;8(1):28-36.

33. Yu G, Lam TT-Y, Zhu H, Guan Y. Two Methods for Mapping and Visualizing Associated Data on Phylogeny Using Ggtree. Mol Biol Evol. 2018 Dec;35(12):3041-3.

34. Paradis E, Schliep K. ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R. Bioinformatics. 2019 Feb 1;35(3):526–8.

35. grid package | R Documentation [Internet]. [cited 2020 Apr 15]. Available from: https://www.rdocumentation.org/packages/grid versions/3.6.2

36. Popovich KJ, Snitkin ES, Hota B, Green SI, Pirani A, Aroutcheva A, et al. Genomic and Epidemiological Evidence for Community Origins of Hospital-Onset Methicillin-Resistant Staphylococcus aureus Bloodstream Infections. J Infect Dis. 2017 01;215(11):1640–7.
37. Camacho C, Coulouris G, Avagyan V, Ma N, Papadopoulos J, Bealer K, et al. BLAST+:
architecture and applications. BMC Bioinformatics. 2009 Dec 15;10(1):421.

38. Wattam AR, Davis JJ, Assaf R, Boisvert S, Brettin T, Bun C, et al. Improvements to PATRIC, the
all-bacterial Bioinformatics Database and Analysis Resource Center. Nucleic Acids Res. 2017
04;45(D1):D535–42.

39. Li H, Durbin R. Fast and accurate short read alignment with Burrows–Wheeler transform.
Bioinformatics. 2009 Jul 15;25(14):1754–60.

40. Liu P, Li X, Luo M, Xu X, Su K, Chen S, et al. Risk Factors for Carbapenem-Resistant Klebsiella
pneumoniae Infection: A Meta-Analyses. Microbial Drug Resistance. 2017 Jul 27;24(2):190–8.

41. Shankar-Sinha S, Valencia GA, Janes BK, Rosenberg JK, Whitfield C, Bender RA, et al. The
Klebsiella pneumoniae O Antigen Contributes to Bacteremia and Lethality during Murine
Pneumonia. Infection and Immunity. 2004 Mar 1;72(3):1423–30.

42. Tedijanto C, Olesen SW, Grad YH, Lipsitch M. Estimating the proportion of bystander selection for
antibiotic resistance among potentially pathogenic bacterial flora. Proceedings of the National
Academy of Sciences. 2018 Dec 18;115(51):E11988–95.

43. Marsh JW, Mustapha MM, Griffith MP, Evans DR, Ezeonwuka C, Pasculle AW, et al. Evolution of
Outbreak-Causing Carbapenem-Resistant Klebsiella pneumoniae ST258 at a Tertiary Care Hospital
over 8 Years. mBio. 2019 Oct 29;10(5):e01945-19.

44. Zhou K, Xiao T, David S, Wang Q, Zhou Y, Guo L, et al. Novel Subclone of Carbapenem-Resistant
Klebsiella pneumoniae Sequence Type 11 with Enhanced Virulence and Transmissibility, China -
Volume 26, Number 2—February 2020 - Emerging Infectious Diseases journal - CDC. [cited 2020
Apr 16]; Available from: https://wwwnc.cdc.gov/eid/article/26/2/19-0594_article

45. Gu D, Dong N, Zheng Z, Lin D, Huang M, Wang L, et al. A fatal outbreak of ST11 carbapenem-
resistant hypervirulent Klebsiella pneumoniae in a Chinese hospital: a molecular epidemiological
study. The Lancet Infectious Diseases. 2018 Jan 1;18(1):37–46.

46. Chapman SJ, Hill AVS. Human genetic susceptibility to infectious disease. Nat Rev Genet. 2012
Figure 1: Infection and anatomic site cluster on the phylogeny. Maximum likelihood phylogenetic tree of all isolates including infection or colonization classification for each isolate and anatomic site of isolation. The scale bar to the right of the phylogeny shows the branch length in substitutions per site. Testing for non-random distribution of isolates on the phylogeny (see supplementary methods) revealed clustering of infection, respiratory, and urinary isolates on the phylogeny, respectively.
Figure 2: Test AUROCs for various classifiers identifying CRKP colonization vs. infection vary substantially across data splits. (A) Test AUROCs for 100 train/test splits used to build models with L2 regularized logistic regression. All isolates from a given LTACH were included in either the training split or the testing split for each data split. We built models using five different feature sets, keeping the same 100 data splits. AUROCs of different feature sets were not significantly different. In the right two panels, the curated genomic feature set AUROCs are compared to: (B) the uncurated genomic feature set AUROCs, and (C) the patient feature set AUROCs. Each point is the resulting pair of AUROCs for models built with the same data split, but the two respective feature sets. The dotted lines in all 3 panels indicate the AUROC for choosing an outcome randomly (0.5); anything below the line is worse than random, and anything above the line is better than random. The solid diagonal line in the right two panels is the line y=x; points below the line correspond to a higher curated genomic AUROC for that data split, and points above the line correspond to a higher uncurated genomic AUROC (B), or patient AUROC (C), respectively. The colors in panels (B) and (C) correspond to the colors in panel (A); the points in a given colored area indicate that that feature set had the higher AUROC for that data split. In both cases, one feature set does not consistently outperform the other (p=0.4; see supplementary methods for p-value calculation). AUROC=area under the receiver operating characteristic curve.
Figure 3: Features consistently associated with colonization or infection sometimes differ between the overall, respiratory, and urinary models. Feature-specific improvement in model performance, measured as the mean difference between test and permuted AUROC (see methods), of features found to be consistently associated with colonization or infection in at least one of the following analyses: overall, respiratory-specific, urinary-specific. We consider features to be associated with infection/colonization if the AUROC difference was greater than zero in over 75% of the 100 data splits. The vertical solid black line indicates a difference of zero (i.e. the feature provides no improvement to model performance). Horizontal dotted lines separate features associated with urinary but not respiratory isolates (top), both urinary and respiratory (or all) isolates (middle), or respiratory but not urinary isolates (bottom). Bla=Beta lactamase, res=confers resistance to that antibiotic class.
Figure 4: Select epidemiologic and genomic features visualized on the phylogeny indicate that a sub-clade of ST258 clade II may exhibit enhanced niche-specific adaptation and virulence. ST258 maximum likelihood phylogeny with the tip labels colored by KPC gene. The blue box indicates the sub-lineage with apparent altered niche-specific adaptation that acquires an additional virulence locus. The heatmap beside the tree indicates information about the isolate. From left to right: if it is a respiratory isolate, if it is an infection isolate, if kfoC is disrupted, and if it contains ICEKp10. Disrupted kfoC was associated with infection in the overall and respiratory machine learning analyses and ICEKp10 presence was associated with infection in the overall analysis. The scale bar to the top left of the phylogeny shows the branch length in substitutions per site. ybt=Yersiniabactin; ybt0 and ybt17 are two ybtSTs defined by Kleborate.