Molecular Epidemiology of ESBL E. coli Over a 2 Year Period in Worcester Massachusetts USA

piperacillin-tazobactam

ceftazidime, cefepime

Only 10% sensitive to

fluoroquinolones

On top is relative

over time while

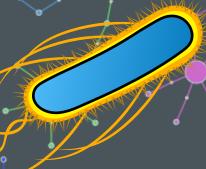
others are transient



UMass Chan



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Abstract

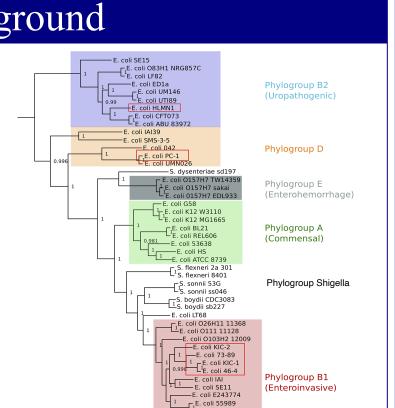
To better understand the population genomics of extended-spectrum beta-lactamase (ESBL) producing Escherichia coli and their associated antibiotic resistance phenotypes, 129 phenotypically identified ESBL isolates collected between 2017-04 to 2018-11 from a single academic medical center underwent whole genome sequencing (WGS) with the presence of antibiotic resistance genes identified by screening against the CARD database. The odds of phenotypic resistance to beta-lactam agents were calculated for each known resistance gene. A novel approach based on k-mer-enrichment among antibiotic resistance phenotypical groups was developed to detect genomic markers associated with phenotypic resistance. The presence of known beta-lactamase CTX-M-15 appears to increase the odds of resistance to key betalactam agents. A novel k-mer-enrichment approach identified a novel marker of piperacillin-tazobactam

Background

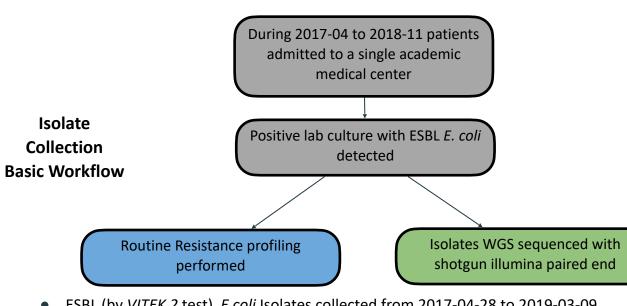
Escherichia coli (E. coli)

- Large diverse group of gram-negative, facultative anaerobic, rod-shaped
- Can be split into different phylogenetic groups that can cause a range of diseases or just be a commensal organism

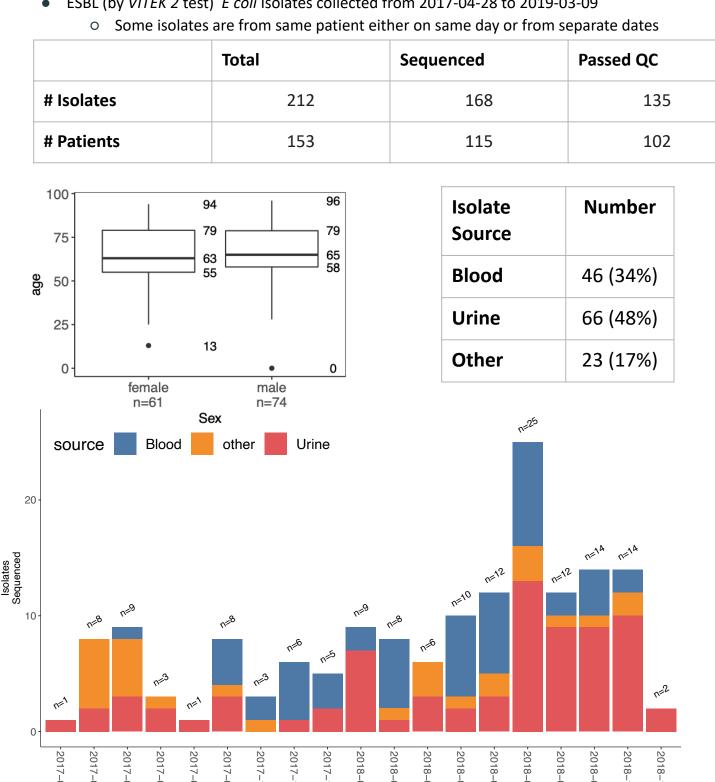
 The emergence of ESBL E. coli is primarily driven by a clonal expansion of one particular strain, Strain Type (ST)131 E. coli which has dominated the expansion since around the year 2000



Methods: Isolate Collection



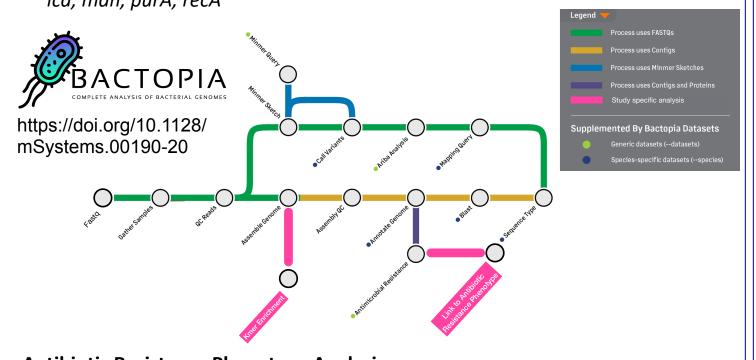
• ESBL (by VITEK 2 test) E coli Isolates collected from 2017-04-28 to 2019-03-09



Methods: Genomic Analysis Antibiotic Resistance Phenotype

Sequence Analysis

- Isolates were sequenced on NextSEQ Illumina 150x2 paired end
- Raw sequences were then analyzed using a Nextflow pipeline Bactopia with some additional study specific analyzes
- See diagram below for all steps but briefly:
- Raw sequences were assembled by Unicycler to create assembled contigs
- Known drug resistance targets were analyzed primarily from CARD database
- Strain typing (ST) done with MLST Achtman schemea using loci adk, fumC, gyrB, icd, mdh, purA, recA

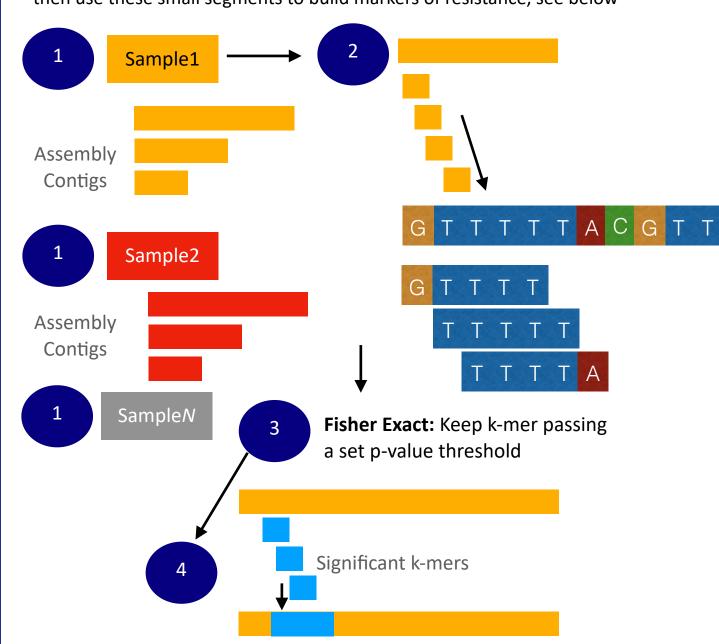


Antibiotic Resistance Phenotype Analysis

- Isolates were sent for routine antibiotic resistance phenotype analysis at local Quest Microbiology lab using standard **VITEX** testing
- Phenotype was then associated with genomic analysis to both known markers of resistance as well as a novel method (see below)

Methods: De-novo detection of markers of antibiotic resistance

- There are many databases of known drug resistance associated genomic markers • In this dataset, antibiotic susceptibility phenotype is also known and therefore not only can known markers can be used to see associations with these phenotypes but also allows for detection for novel markers of resistance
- To accomplish this novel detection, a method was developed to look for small segments of DNA called k-mers that had significant increased odds of resistance to antibiotics and then use these small segments to build markers of resistance, see below



1) Take all assemblies for each sample with associated antibiotic resistance profiles 2) Break each contig into small sub-strings called k-mers

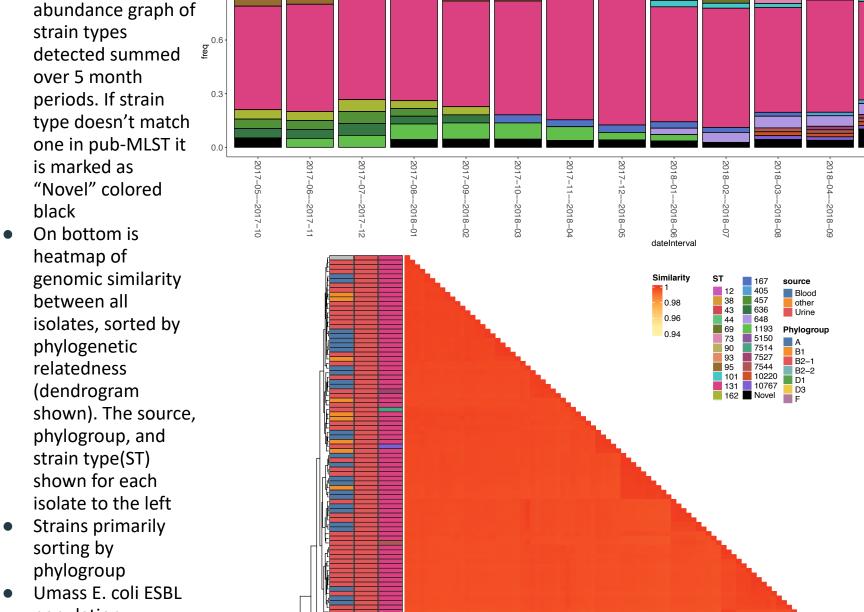
- 3) Create 2x2 table of presence/absence of k-mer and resistance/sensitivity to antibiotic and calculate odds of resistance using Fisher exact test, filter to p-value threshold (for this study used p-value <0.001)
- 4) Merge overlapping k-mers with significant increase in odds of resistance to determine sub regions associated with resistance

Figure represents analysis with k-mers into 5-mers, for this study we used 19-mers

profiles of all sequenced isolates. Each row is an isolate, each column is an **antibiotic**, **red** is resistant and green is sensitive, bars on top of column have percent sensitive Depending on collection date and source different antibiotics were tested, if an antibiotic wasn't tested it is blank Virtually all strains are resistant to ceftriaxone ampicillin, and cefazolin but only a subset are also resistant to ampicillin-sulbactam

Results: Antibiotics Profiles

Results:Population Structure

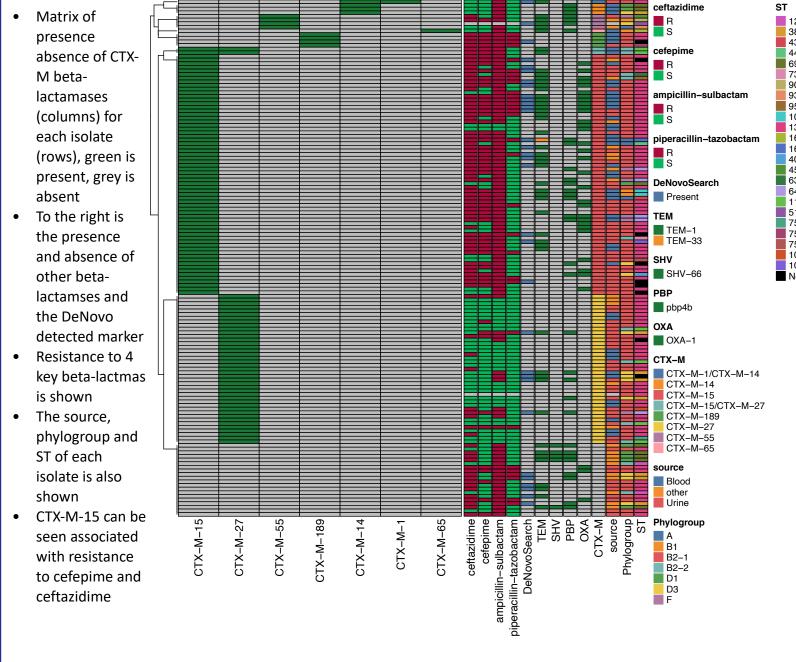


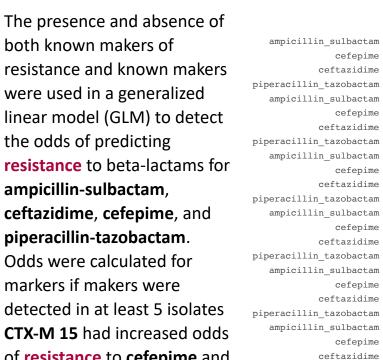
population dominated by ST131 which are highly related with several novel strain branching from ST131 remains dominant over the months of collections with some other minor strains persistent

Results Novel Markers Detection

Our k-mer resistance search approach was able to identify the known genes listed above and also identified a 1374 bp open reading frame with unknown function that had increased odds of resistance to piperacilling tazobactam resistance (3-23.8, p<0.001). This 1374 bp open reading frame did not share any homology with known beta-lactamases. Protein had a 99% match to proteins labeled as DUF262 domain-containing protein in NCBI database. The structure was predicted using Alpha-Fold and existing structures searched via PDBeFold v2.59 with closet homology matched a plasmid mobility element at 40%. This could be suggestive of possible plasmid related protein or mobility element itself

Results: Beta Lactamases

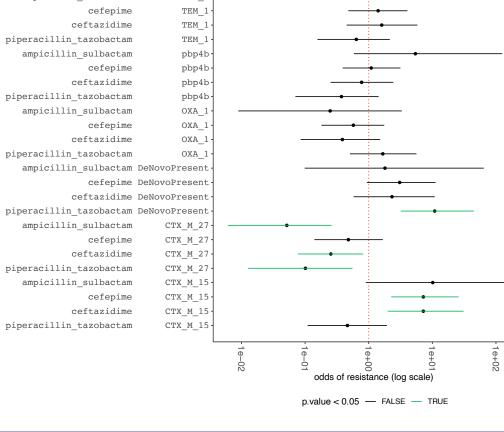




CTX-M 15 had increased odds of **resistance** to **cefepime** and ceftazidime The DeNovo marker had

increased odds to resistance

to piperacillin-tazobactam



Conclusions

- Umass population of *E. coli* ESBL similar to the world with a ST131 dominated population. Population of **ST131** appears stable over time with minor strains persistent or become transient
- CTX-M-15 was the only known marker to show significant increased odds of resistance to key additional beta-lactams of cefepime and ceftazidime
- Our de-novo k-mer phenotypical search approach can be applied to any phenotype or grouping to detect new genomic markers associations
- This analysis was able to detect a new potential marker of resistance to piperacillin-tazobactam, the exact mechanism for the association is currently under investigation

References

- 1. Ward, Doyle V., Andrew G. Hoss, Raivo Kolde, Helen C. van Aggelen, Joshua Loving, Stephen A. Smith, Deborah A. Mack, et al. 2019. "Integration of Genomic and Clinical Data Augments Surveillance of Healthcare-Acquired Infections." Infection Control and Hospital
- Epidemiology: The Official Journal of the Society of Hospital Epidemiologists of America 40 (6): 649-55. 2. Petit, Robert A., 3rd, and Timothy D. Read. 2020. "Bactopia: A Flexible Pipeline for Complete Analysis of Bacterial Genomes." mSystems 5 (4). https://doi.org/10.1128/mSystems.00190-20.