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Distribution of β-Lactamase Resistance Genes and Lineage Background in Clinical Isolates Producing Extended-Spectrum β-Lactamases by High-Resolution Genomic Analysis

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SENTRY ANTIMICROBIAL SURVEILLANCE

Introduction

- Dramatic cost reductions and increased quality of whole genome sequencing (WGS) during recent years have made this technology economically feasible, not only for scientific research but also for clinical diagnostics and surveillance programs
- The power of genome sequencing, bioinformatic tools, and curated databases for *in silico* analysis can provide organism identification, a better understanding of antimicrobial resistance, virulence genes, and epidemiology of bacterial populations
- The combination of WGS and proprietary bioinformatic tools was used to screen for β-lactamase genes and to determine the epidemiology of *Escherichia coli* and *Klebsiella pneumoniae* producing extended-spectrum β-lactamases (ESBLs) and/or carbapenemases, causing bloodstream (BSIs) and urinary tract (UTIs) infections in patients hospitalized in the United States

Materials and Methods

Bacterial isolates

- A total of 3,525 isolates (2,751 *E. coli* and 774 *K. pneumoniae*) causing BSIs (n=892) and UTIs (n=2,633) in hospitalized patients in the United States were collected as part of the SENTRY Antimicrobial Surveillance Program
  - These isolates were consecutively collected (1 per patient) from 81 sites located in 36 states in 9 US census divisions and were submitted to JMI Laboratories (North Liberty, Iowa, USA) as part of the SENTRY Program for 2016
- Isolates were initially identified by the participating laboratory, and identifications were confirmed at JMI Laboratories using matrix assisted laser desorption ionization time of flight technology mass spectrometry (Bruker Daltonics, Bremen, Germany) and genome sequencing

Antimicrobial susceptibility testing

- Isolates were tested for susceptibility by broth microdilution using frozen-form broth microdilution panels containing cation-adjusted Mueller-Hinton broth and manufactured by JMI Laboratories according to CLSI M07 (2018)
  - Isolates displaying elevated MIC results (≥2 mg/L) for ceftriaxone, aztreonam, ceftazidime, or imipenem/meropenem (MIC, ≥2 mg/L) were selected for further characterization

Screen for β-lactamase genes by WGS and bioinformatic tools

- Total genomic DNA was extracted using the Thermo Scientific™ KingFisher™ Flex Magnetic Particle Processor (Cleveland, Ohio, USA)
- DNA samples were quantified using the Qubit™ High Sensitivity DS-DNA assay (Invitrogen, ThermoFisher Inc.) and normalized to 0.2 ng/μL
  - A total of 1 ng high-quality genomic DNA was used as input material for library construction
- DNA libraries were prepared using the Nextera XT™ library construction protocol (Illumina, San Diego, California, USA) following the manufacturer's instructions and sequenced on a MiSeq Sequencer (Illumina) at JMI Laboratories
- Each raw sequencing data set was quality assured, error corrected, and assembled *de novo* using the SPAdes genome assembler
- Assembled genomes were subjected to a proprietary software (JMI Laboratories) to screen for known β-lactamase-encoding genes

Epidemiology typing

- Multilocus sequence typing (MLST) was performed by extracting from assembled genomes previously defined sets of 7 housekeeping gene fragments (~500 bp)
  - Each fragment was compared to known allele variants for each locus (housekeeping gene) on the MLST website (http://www.mlst.net)
- An allele sharing 100% genetic identity with a known variant received a numeric designation, and a 7-number sequence (1 for each housekeeping gene) formed an allelic profile, defined as sequence types (STs)
  - ST profiles sharing 100% genetic identity in at least 6 of 7 MLST loci were grouped into a clonal complex (CC) named after its presumed ancestral genotype by eBurst analysis
  - Isolates containing alleles that did not match an existing sequence in the MLST database were submitted/deposited for allele and ST assignments

Results

- A total of 11.6% and 16.1% of *E. coli* causing UTI and BSI, respectively, met the MIC-based criteria, while 11.0% and 13.7% of *K. pneumoniae* isolates causing UTI and BSI, respectively, met the MIC-based criteria (data not shown)
- Among *E. coli* isolates that met the MIC-based screening criteria, bla_{CTX-M} variants (87.6% overall) represented the most common ESBL genes in which CTX-M group 1 (60.5% overall) and group 9 (26.9% overall) prevailed (Figure 1A)
- A total of 60.3% of *K. pneumoniae* isolates carried bla_{CTX-M} variants, and 52.7% and 7.6% of those isolates carried CTX-M genes of group 1 and 9, respectively (Figure 1B)
- A small number of *E. coli* (n=2; 0.6%) and *K. pneumoniae* (n=13; 12.9%) clinical isolates harbored *Klebsiella pneumoniae* carbapenemase (KPC)-encoding genes; all but 2 *K. pneumoniae* were recovered from urine (Figures 1A and 1B)
- Nineteen and 43 unique STs were noted in *E. coli* causing BSI and UTI, respectively, and ST131 was the most common CC among BSI (53.6%) and UTI (58.2%) *E. coli* isolates (Figure 2)
- A total of 27 and 29 STs were noted among *K. pneumoniae* isolates causing BSI and UTI, respectively. CC11 (28.3%; 16/17 isolates were ST258) prevailed in UTI pathogens, while CC307 (15.0%) was the most common CC among BSI isolates (Figure 3)
- Among *E. coli* isolates, the majority of $bla_{CTX-M-15}$ (70.3%) were detected within ST131 isolates, with similar occurrences among those causing BSI (68.4%) or UTI (71.1%) (data not shown)
- Most ST131 *E. coli* isolates causing BSI carried bla_{CTX-M} genes belonging to the CTX-M group 1 (75.0%) and a small percentage of genes belonged to the group 9 (17.3%) (Figure 4A)
- ST131 *E. coli* isolates causing UTI had a greater number of bla_{CTX-M} genes belonging to the CTX-M group 9 (30.4%) when compared with isolates causing BSI (17.3%) (Figures 4A and 4B)

Figure 2 Distribution of clonal complexes among *E. coli* isolates recovered from UTI (outer circle) and BSI (inner circle)

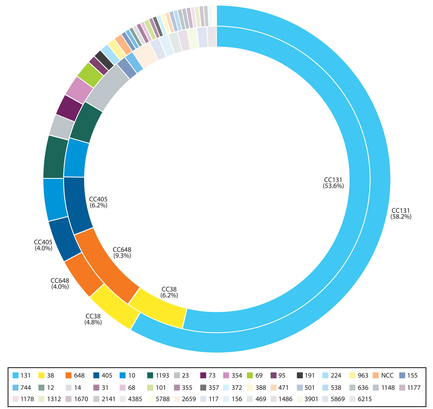


Figure 3 Distribution of clonal complexes among *K. pneumoniae* isolates recovered from UTI (outer circle) and BSI (inner circle)

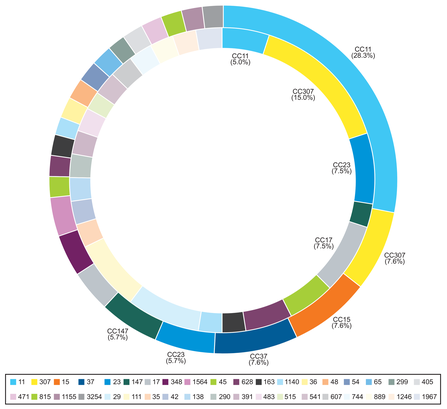


Figure 1 Distribution of β-lactamase-encoding genes detected in *E. coli* (1A) and *K. pneumoniae* (1B) causing UTI or BSI

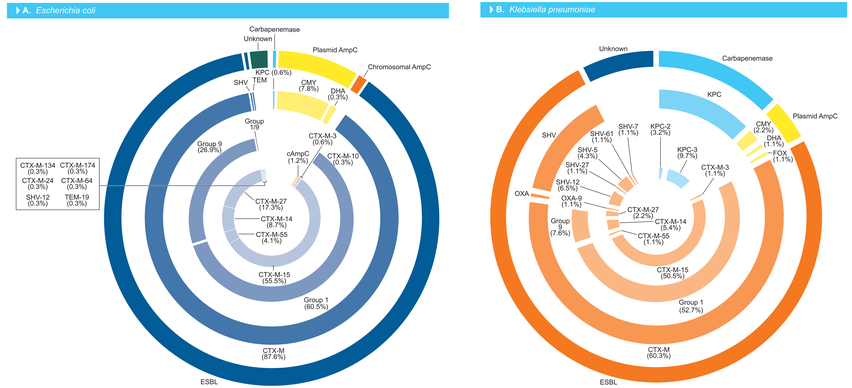
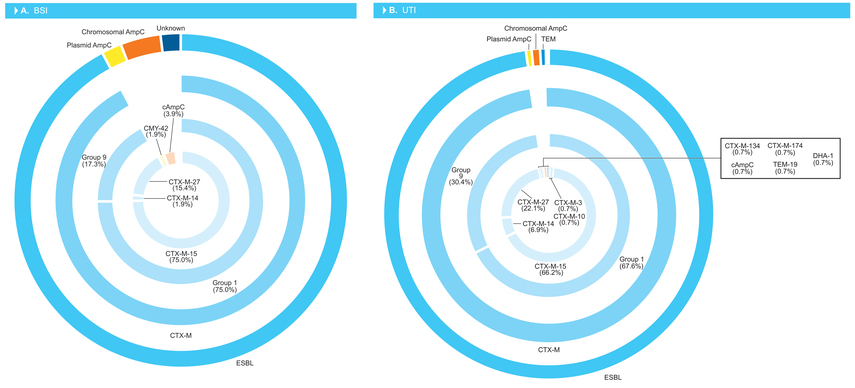


Figure 4 Distribution of β-lactamase-encoding genes detected in *E. coli* ST131 causing UTI or BSI



Conclusions

- These data show the dissemination of *E. coli* ST131 isolates in the United States, regardless of infection site; the population structure of *K. pneumoniae* was more heterogeneous
- In addition, the data confirm the dominance of group 1 CTX-M-encoding genes among ST131 *E. coli*; however, the data presented here may suggest emergence of genes associated with CTX-M of group 9 among UTI isolates
- This study provides a benchmark for the distribution of β-lactamase genes and the population structures of the most common *Enterobacteriaceae* species responsible for BSI and UTI in US medical centers in the SENTRY Program
- Coupling genome sequencing with powerful bioinformatic tools can be useful to help detect and monitor resistance genes and bacterial populations in surveillance programs
- Future studies that include isolates recovered from US medical centers during multiple years can provide a trend analysis for a better understanding of the dissemination of resistance genes and evolution of bacterial populations

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