Background: Phenotypic antimicrobial susceptibility testing (AST) is the standard guide for antibiotic use, but it takes days to perform. Molecular tests can rapidly detect resistance genes within hours of specimen collection, but they have not demonstrated comprehensive prediction of phenotypic antibiotic resistance.

Material/Methods: We compared PCR results for 126 resistance genes with phenotypic AST results across 1931 highly resistant clinical isolates of *E. coli* including 1366 ESBLs collected at the Intermountain Medical Center over 2008 to 2015 plus 565 *E. coli* isolates from Merck’s SMART Surveillance Network as supplied by International Health Management Associates. Statistical models were used to predict antibiotic resistance from resistance genes.

Results: We identified a panel of critical resistance genes and developed statistical algorithms to accurately predict phenotypic resistance for several antibiotics across 1931 highly resistant clinical isolates of *E. coli*. The study demonstrates rapid genotypic prediction of resistance for 17 antibiotics representing 7 antibiotic classes as comprehensive information toward antibiotic options against a spectrum of resistance. Future studies will improve prediction of carbapenem resistance through evaluation of more *E. coli* isolates.

Conclusions: Molecular detection of resistance genes offers the potential to predict antibiotic resistance days ahead of phenotypic AST for Gram-negative bacteria toward better patient and hospital management.