Renal Transplant Recipient Resistomes Reveal Expansive Sub-Clinical Burden of Resistance After Treatment for ESBL-Producing Bacterial Infections.

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Background: Renal transplant recipients have frequent infection and colonization with antibiotic resistant (AR) bacteria. However, little is known about the burden of AR following targeted antibiotic treatment.

Methods: This was a prospective study conducted as part of a single center clinical trial at Emory University. Demographic and clinical data regarding transplant and AR bacterial infection were abstracted. Stool samples were collected from renal transplant recipients treated with antibiotics for Extended-Spectrum β-Lactamase (ESBL)-producing gram negative infections. Bacterial cultures with AR-selective and differential media and Illumina short-read sequencing were performed on stool sample nucleic acid extracts. Confirmatory phenotypic isolate AR testing was performed with the Vitek2 platform. Resistome profiles were produced by assembling short reads using MetaSPAdes, predicting protein sequences using Prodigal and classifying proteins as antimicrobial resistance determinants using AMRFinderPlus. Patient results were compared to stool metagenomes from matched Human Microbiome Project healthy controls.

Results: Metagenome sequencing was performed for 8 patient stool samples from 6 patients (5 female) and compared to metagenome sequence data from 18 matched Human Microbiome Project healthy controls. Patient stools were collected a median of 30 days after infection. The median number of AR genes per patient metagenome was 48.5 (range 23 to 87 genes). The median number of AR genes per control metagenome was 24 (range 16 to 25 genes). We detected 128 unique AR genes across all samples, 65% of which were detected in patient samples but not controls. All AR genes found in control metagenomes were present in at least one patient metagenome. No AR genes detected in patients were common to all patients. Subsets of clinically relevant genes corresponded with patient stool AR bacteria culture results.

Conclusion: Viable AR bacteria and diverse AR gene profiles were frequently detected from renal transplant recipient stool samples after antibiotic treatment for infection. These data suggest that AR bacterial colonization and AR gene profiles may require distinct treatments other than systemic antibiotics for eradication.

Figures

A: Violin plot of alpha diversity (Shannon Index) of Antimicrobial Resistance Genes (ARG) in fecal samples from renal transplant recipients treated for ESBL infections. Shannon diversity was not statistically significantly different from matched HMP controls.

B: Multidimensional scaling (MDS) plot of beta diversity (Bray-Curtis dissimilarity) of Antimicrobial Resistance Genes (ARG) in fecal samples from renal transplant recipients treated for ESBL infections. MDS plot shows between-sample clustering of antibiotic-treated renal transplant recipients (yellow) compared to HMP controls (purple).

C: Linear Discriminant Analysis (LDA) scores from LefSe analysis of differentially abundant Antimicrobial Resistance Genes (ARG) in fecal samples from renal transplant recipients treated for ESBL infections.

D: Heatmap of normalized abundance of Antimicrobial Resistance Genes (ARG) as Log_2 reads per kilobase per million (RPKM). Metadata rows show selective MDRO culture results for fecal samples from renal transplant recipients treated for ESBL infections. Metadata column shows class of ARG.

E: Line plots of normalized abundance of Antimicrobial Resistance Genes (ARG) as reads per kilobase per million (RPKM) showing patient-specific expansion of ARG abundance after antibiotic treatment for ESBL-producing bacterial infections in renal transplant recipients.