JOURNAL OF UNDERGRADUATE RESEARCH

Antimicrobial Resistance Gene Carriage is not Associated with Uremic Toxin Levels in Chronic Kidney Disease Patients

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Abstract

In chronic kidney disease (CKD), uremic toxin molecules build up and risk of infection increase overtime, making the presence of antimicrobial resistant (AMR) bacteria in the gut more problematic with advancing disease. This study assessed if serum levels of uremic toxins and gut carriage of AMR genes are correlated in individuals with CKD. Whole metagenomic sequencing of stool samples and measured levels of 4 uremic toxins from 21 individuals with CKD were obtained. Multivariable, multivariate linear regression was performed with total abundance of AMR genes and abundance of specific drug classes of AMR genes as outcome variables and serum levels of four uremic toxins as predictor variables. Beta diversity differences of AMR genes were visualized using non-metric multidimensional scaling (NMDS) based on robust Aitchison distances and tested using PERMANOVA. There were no significant associations between uremic toxin levels and AMR gene abundance, or with any of the drug classes of AMR genes. There were no significant differences in beta diversity of AMR genes. These null findings suggest that increased uremic toxin levels are not associated with an increase in AMR carriage, and therefore uremia does not appear to be a direct cause of increased resistant bacteria in patients with more severe CKD.

Keywords: antimicrobial resistance, chronic kidney disease, gut microbiome

Introduction

The human gut microbiome refers to the genetic information for the microbiota, the diverse community of microorganisms that inhabit the intestines (Turnbaugh et al., 2007). The human gut microbiota aid in the breakdown of certain foods for nutrients that humans do not digest well, create a barrier to prevent pathogens from easily spreading throughout the body, convert primary bile acids to secondary bile acids to act as hormones, and play a role in immunity(Al Khodor & Shatat, 2017; Chi et al., 2021). Gut microbiome composition, including abundance and diversity, can differ between healthy individuals due to factors such as genetics, diet, geographic location, (Ursell et al., 2012) and antibiotic use, but an individuals' specific composition typically looks similar over longer periods of time (5-10 years) (Faith et al., 2013).

Antimicrobial resistance (AMR) can develop in gut microbiota when taking antimicrobial medications to fight infections (O'Neill, 2016). As resistance develops, medications previously capable of slowing or stopping bacteria growth cease to impact microbial growth (O'Neill, 2016). AMR is a serious global public health problem. Organisms that develop AMR were the direct cause of 1.27 million deaths in 2019, with 4.95 million deaths being associated with AMR around the world in that same year (Murray et al., 2022). The duration of hospital care and death occurrences are higher in patients with resistant infections than in non-resistant infections (Wang et al., 2019). Patients can also acquire antimicrobial resistant bacteria from other patients in clinical settings, termed a nosocomial infection (Pop-Vicas et al., 2008). Commensal microorganisms with AMR are primarily found in the human gut (Anthony et al., 2021). Horizontal gene transfer enables AMR genes to be transferred between commensal bacteria or from commensal bacteria to pathogens enabling their resistance (McInnes et al., 2020). An individual's carriage of AMR genes in the gut microbiome can be detected and assessed from stool samples.

In addition to its role in AMR, the gut microbiota are associated with chronic kidney disease (CKD). Research has suggested associations between CKD and gut dysbiosis, including alterations in gut microbiota composition and function (Ren et al., 2020). The Kidney Disease Improving Global Outcomes (KDIGO) group defines chronic kidney disease as abnormalities of kidney structure or function for at least 3 months (Group, 2013). With this condition, the malfunctioning kidney's inability to filter out these waste compounds to a healthy level (Levin et al., 2017) and inability to completely remove the toxins during dialysis treatment (Lim et al., 2021) causes a buildup of uremic toxin molecules in the body which are damaging to many of the body's organs (Lisowska-Myjak, 2014). The relationship between gut microbiome dysbiosis and chronic kidney disease appears to be bidirectional. The accumulation of uremic toxins, which are no longer being properly filtered out by the kidney, in the intestine, promote the colonization of urea-consuming microbes not seen in healthy individuals. Prescribed alterations to diet after being diagnosed with CKD, including reduced intake of protein, sodium, potassium, and phosphate, play a significant role in alterations in the microbiome. Additionally, individuals with CKD may experience gut dysbiosis due to disease-related factors including, "decreased consumption of dietary fiber and increased constipation, impaired protein assimilation, frequent

use of antibiotics, and iron therapy," (Armani et al., 2017 p. 2). In the other direction, alterations in the gut worsen symptoms of CKD.

Certain gut microbes produce the uremic toxins trimethylamine-N-oxide, indoxyl sulfate, and *p*-cresyl sulfate, and phenylacetylglutamine that continue to damage renal function and other tissues by activating inflammatory pathways (Feng et al., 2021) and are associated with cardiovascular disease risk. *P*-cresol is a byproduct formed by gut microbiota metabolism of tyrosine and phenylalanine, and is quickly further metabolized to *p*-cresyl sulfate (CS) (Evenepoel et al., 2009). An increase in CS levels, as happens during CKD, has been associated with increased oxidative stress in leukocytes and in human vascular smooth muscle cells, endothelial damage, cardiovascular morbidity and mortality seen in CKD patients, insulin resistance, and immune system suppression (Gryp et al., 2017). Gut microbial metabolism of tryptophan forms indole which is further metabolized to indoxyl sulfate (IS) in the liver (Evenepoel et al., 2009). Increased levels of IS are associated with increased oxidative stress, reduced antioxidant capacity and increased inflammation, damage to renal tubular cells, and cardiovascular events (Cheng et al., 2020). Phenylacetylglutamine (PAG) is a byproduct of gut microbial metabolism of phenylalanine, and a high serum level of PAG is a risk factor for overall mortality and cardiovascular disease (Poesen et al., 2016). Trimethylamine is formed through gut bacteria metabolism of choline, phosphatidylcholine, betaine, and carnitine, or from consuming foods already containing trimethylamine or trimethylamine N-oxide (Pelletier et al., 2019). The compound is converted to trimethylamine N-oxide (TMAO) by the liver (Pelletier et al., 2019). Increased serum levels of TMAO is associated with increased risk of cardiovascular events (Pelletier et al., 2019).

Individuals with CKD are also at increased risk for overall infection (Cohen, 2020) and acquiring nosocomial drug-resistant bacteria (Calfee, 2015). Uremic toxin buildup likely leads to suboptimal white blood cell functioning which increases risk of infection in CKD patients (Cohen, 2020). Concerningly, infections are the second most common cause of death in CKD patients (Group, 2013). With more infections, CKD patients tend to be given more antibiotics and are in the hospital more where they are at increased risk of acquiring nosocomial drug-resistant bacteria (Calfee, 2015). As a result, CKD patients experience more AMR infections and colonization than the general population (Wang et al., 2019). The association between increased

antibiotic use and increased uremic toxin levels may also result in increased carriage of antimicrobial resistant organisms in the gut microbiota. In short, the relationship between CKD, uremic toxins, and the gut microbiome are bidirectional and complex, with the potential to alter the resistome because of both gut microbiome composition changes and increased need for antibiotics due to infection. This study seeks to explore if gut microbiota carriage of AMR genes is increased with higher levels of uremic toxins. If this association exists, it suggests that microbiome targeted interventions for CKD patients may matter for resistant infection risk.

Methods

Analysis of AMR gene abundance and beta diversity of AMR genes were performed using metagenomic data and serum uremic toxin levels collected in a previous study, making this work a secondary analysis of existing data sets. Only deidentified data were used for this study.

Sample Collection, DNA Extraction, and Sequencing

Serum uremic toxin level measurements, fecal sample collection, DNA extraction, metagenomic sequencing, and data trimming were performed in a previous study led by the Dahl lab at the University of Florida. Stool samples were collected from 21 patients receiving hemodialysis treatments at one hemodialysis center in Florida. As reported previously (Taft et al., 2024), stool collection was done using Fisherbrand™ Commode Specimen Collection System (Fisher Scientific, cat. no. 02-544-208). Samples were kept on ice for up to 6 h until processing, and stored at -80°C for later DNA extraction. Pre-dialysis blood samples were collected from the participants after one day of no dialysis treatments. Levels of the uremic toxin molecules indoxyl sulfate (IS), *p*-cresyl sulfate (CS), trimethylamine-*N*-oxide (TMAO), phenylacetylglutamine (PAG) were measured using Liquid Chromatography–Mass Spectrometry (LC-MS) as described by Fatani et al.(Fatani et al., 2023). DNA from the collected stool samples was extracted using the Qiagen AllPrep PowerFecal DNA/RNA kit, quantified using the QuBit dsDNA BR Assay kit, and sequenced by UF ICBR using Illumina NovaSeq S4 150 PE chemistry. Host subtraction was performed using BMTagger (Rotmistrovsky & Agarwala, 2011) and sequences were quality trimmed using Trimmomatic (Bolger et al., 2014).

AMR Gene Identification, Abundance Normalization, and Abundance Regressions Analysis

The sequences were inputted into the AMR++ bioinformatic pipeline which referenced the MEGARes 3 (Bonin et al., 2023) database to identify antimicrobial resistance genes present in the sample and output counts of how many of each gene were present. The abundance of each AMR gene was then normalized by gene length and the number of 16S rRNA gene sequences detected. This normalization was to prevent genes that were twice as long from being detected as twice as abundant than shorter genes.

Multivariable, multivariate regressions were performed with total abundance of AMR genes and abundance of specific drug classes of AMR genes as outcome variables and serum levels of the 4 uremic toxins CS, IS, PAG, and TMAO as predictor variables in R Studio (Team, 2022).

AMR Gene Beta-Diversity Analysis

Beta diversity differences by AMR genes were visualized using non-metric multidimensional scaling (NMDS) based on robust Aitchison's distance in the R vegan package(Oksanen et al., 2007), and data points were colored by uremic toxin level. Differences in AMR gene beta-diversity by uremic toxin molecules were tested using PERMANOVA as implemented in the adonis2 function in the vegan package (Oksanen et al., 2007).

Results

Multivariable, Multivariate Linear Regression Testing Total Abundance of AMR Genes vs Uremic Toxin Levels Show No Significant Association After Correction for Multiple Comparisons

There were 306 AMR genes detected in the samples by AMR++ that were used as outcome variables in a multivariate, multilinear regression with uremic toxin levels of CS, IS, PAG, and TMAO as predictor variables. As shown in Table 1, 34 genes were associated with uremic toxin levels prior to multiple comparison correction. These 34 genes encoded for Aminoglycoside O-phosphotransferases, Ambler class A beta-lactamases, Ambler class C beta-lactamases, Ambler class D beta-lactamases, chloramphenicol acetyltransferases, Aminoglycoside N-acetyltransferases, ABC efflux pumps, tetracycline ribosomal protection

protein, and 23S rRNA methyltransferases. The beta-coefficient was negative for 16 of these associations. No individual gene was significant after Bonferroni adjustment for multiple comparisons.

Table 1. Genes significantly associated with at least one uremic toxin molecules prior to correction for multiple comparisons. Model included all 306 AMR genes as outcomes and all uremic toxin molecules as predictors. With correction, p-value would need to be less than 0.0001 to be significant.

	Beta-Coefficient CS (p-value)	Beta-Coefficient IS (p-value)	Beta-Coefficient PAG (p-value)	Beta-Coefficient TMAO (p-value)
ADPH2-DPRIME, Aminoglycoside O-phosphotransferase (MEG1018)	**5.70E-08 (0.027)**	-4.22E-07 (0.42)	6.10E-07 (0.59)	3.13E-07 (0.77)
BLAEC, Ambler class C beta-lactamase (MEG1275)	**2.78E-07 (0.027)**	-2.06E-06 (0.42)	2.97E-06 (0.59)	1.53E-06 (0.77)
BLAEC, Ambler class C beta-lactamase (MEG1277)	**2.27E-07 (0.027)**	-1.68E-06 (0.42)	2.43E-06 (0.59)	1.25E-06 (0.77)
CAT, chloramphenicol acetyltransferase (MEG1553)	-8.45E-08 (0.32)	**-4.44E-06 (0.026)**	6.14E-06 (0.14)	4.33E-06 (0.26)
CBLA, Ambler class A beta-lactamase (MEG1636)	3.30E-07 (0.47)	-4.81E-06 (0.63)	-2.69E-05 (0.22)	**5.20E-05 (0.020)**
CBLA, Ambler class A beta-lactamase (MEG1637)	3.21E-09 (0.99)	-4.53E-06 (0.63)	-2.03E-05 (0.32)	**4.06E-05 (0.047)**
AAC6-PRIME, Aminoglycoside N-acetyltransferase (MEG212)	**2.10E-07 (0.046)**	1.09E-06 (0.62)	-4.18E-06 (0.38)	1.72E-06 (0.70)
AAC6-PRIME, Aminoglycoside N-acetyltransferase (MEG213)	**3.19E-07 (0.046)**	1.66E-06 (0.62)	-6.35E-06 (0.38)	2.62E-06 (0.70)

AAC6-PRIME, Aminoglycoside N-acetyltransferase (MEG316)	-4.26E-09 (0.95)	-2.88E-06 (0.09)	-2.27E-06 (0.52)	**8.34E-06 (0.023)**
AAC6-PRIME, Aminoglycoside N-acetyltransferase (MEG352)	9.20E-09 (0.78)	-1.33E-06 (0.080)	-1.11E-06 (0.48)	**4.32E-06 (0.0096)**
AAC6-PRIME, Aminoglycoside N-acetyltransferase (MEG364)	1.67E-08 (0.78)	-2.42E-06 (0.080)	-2.00E-06 (0.48)	**7.83E-06 (0.0096)**
MEFA, ABC efflux pump (MEG3805)	6.40E-09 (0.88)	1.33E-06 (0.16)	**-5.38E-06 (0.014)**	2.75E-06 (0.16)
MEFA, ABC efflux pump (MEG3825)	4.27E-09 (0.88)	8.85E-07 (0.16)	**-3.59E-06 (0.014)**	1.84E-06 (0.16)
OXA, Ambler class D beta-lactamase (MEG4750)	3.27E-07 (0.52)	1.72E-05 (0.14)	**-5.20E-05 (0.044)**	7.71E-06 (0.74)
PATA, ABC efflux pump (MEG5397)	7.20E-09 (0.88)	1.49E-06 (0.16)	**-6.06E-06 (0.014)**	3.10E-06 (0.16)
PATB, ABC efflux pump (MEG5398)	4.56E-09 (0.88)	9.45E-07 (0.16)	**-3.83E-06 (0.014)**	1.96E-06 (0.16)
RLMA , 23S rRNA methyltransferase (MEG6057)	4.59E-09 (0.88)	9.52E-07 (0.16)	**-3.86E-06 (0.014)**	1.98E-06 (0.16)
TET32, tetracycline ribosomal protection protein (MEG6969)	1.93E-07 (0.17)	-5.50E-06 (0.080)	6.64E-06 (0.31)	**1.61E-05 (0.018)**
TET40, tetracycline ribosomal protection protein (MEG6992)	3.35E-07 (0.36)	-1.01E-05 (0.22)	-1.40E-05 (0.42)	**4.02E-05 (0.024)**
TET44, tetracycline ribosomal protection protein (MEG6997)	6.67E-07 (0.25)	-1.87E-05 (0.15)	-2.81E-05 (0.30)	**7.36E-05 (0.010)**

TET44, tetracycline ribosomal protection protein (MEG6998)	6.38E-07 (0.31)	-2.18E-05 (0.13)	-2.94E-05 (0.33)	**8.23E-05 (0.0092)**
TETA46, ABC efflux pump (MEG7035)	8.59E-09 (0.95)	4.41E-06 (0.14)	**-1.66E-05 (0.015)**	6.51E-06 (0.28)
TETB46, ABC efflux pump (MEG7053)	2.73E-08 (0.80)	3.74E-06 (0.12)	**-1.39E-05 (0.013)**	5.95E-06 (0.23)
TETM, tetracycline ribosomal protection protein (MEG7104)	3.27E-08 (0.68)	2.68E-06 (0.14)	**-8.16E-06 (0.046)**	1.65E-06 (0.65)
TETM, tetracycline ribosomal protection protein (MEG7105)	2.54E-08 (0.70)	2.29E-06 (0.13)	**-7.32E-06 (0.030)**	1.92E-06 (0.52)
TETM, tetracycline ribosomal protection protein (MEG7115)	1.13E-07 (0.079)	2.09E-06 (0.13)	**-8.36E-06 (0.010)**	4.07E-06 (0.16)
TETM, tetracycline ribosomal protection protein (MEG7126)	1.15E-07 (0.091)	2.28E-06 (0.12)	**-6.75E-06 (0.042)**	1.05E-06 (0.72)
TETM, tetracycline ribosomal protection protein (MEG7134)	5.95E-09 (0.88)	1.23E-06 (0.16)	**-5.01E-06 (0.014)**	2.56E-06 (0.16)
TETM, tetracycline ribosomal protection protein (MEG7140)	1.05E-07 (0.11)	2.55E-06 (0.083)	**-8.24E-06 (0.014)**	2.19E-06 (0.45)
TETM, tetracycline ribosomal protection protein (MEG7146)	**1.20E-07 (0.046)**	6.23E-07 (0.62)	-2.39E-06 (0.38)	9.84E-07 (0.70)
TETO, tetracycline ribosomal protection protein (MEG7152)	1.05E-07 (0.74)	-1.11E-05 (0.12)	-3.12E-06 (0.84)	**3.05E-05 (0.042)**

TETO, tetracycline ribosomal protection protein (MEG7160)	2.26E-08 (0.92)	**-1.11E-05 (0.044)**	4.86E-06 (0.66)	1.96E-05 (0.080)
TETO, tetracycline ribosomal protection protein (MEG7169)	**7.87E-08 (0.046)**	4.09E-07 (0.62)	-1.57E-06 (0.38)	6.47E-07 (0.70)
AAC6-PRIME, Aminoglycoside N-acetyltransferase (MEG75)	1.29E-08 (0.78)	-1.86E-06 (0.080)	-1.55E-06 (0.48)	**6.04E-06 (0.0096)**

Multivariable, Multivariate Linear Regression Testing Drug Class Abundance vs Uremic Toxin Levels Shows No Significant Association

AMR genes from 16 drug classes were detected in the samples. The classes were aminoglycosides, bacitracin, betalactams, cationic antimicrobial peptides, fluoroquinolones, fosfomycin, glycopeptides, metronidazole, MLS (macrolide-lincosamide-streptogramin antibiotics), multi-drug resistance, mupirocin, nucleosides, phenicol, sulfonamides, tetracyclines, and trimethoprim. None of the individual models had an F-statistic p-value less than 0.05, suggesting that no individual model improved on the null model. Table 2 summarizes the individual univariate models. There were no significant associations between uremic toxin levels and the abundance of drug classes of AMR genes after accounting for multiple comparisons.

Table 2. Drug class by uremic toxin model results. As 16 models were constructed, a p-value less than 0.003 was necessary for significance.

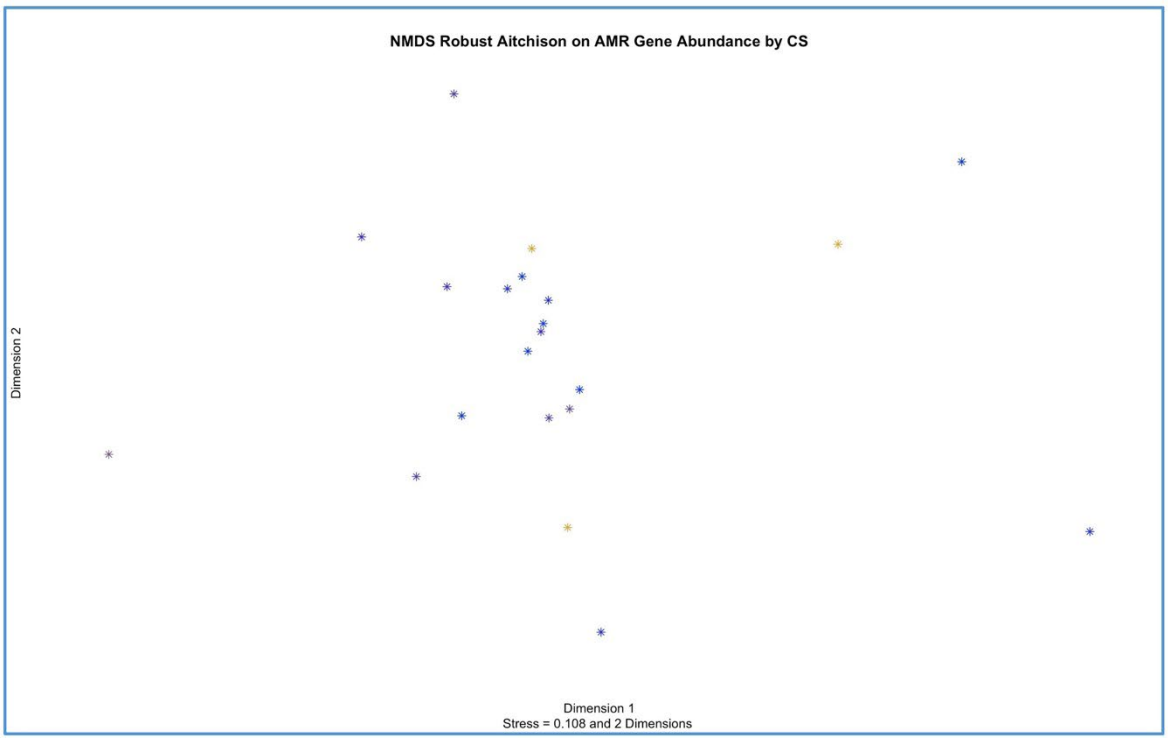
	Beta-Coefficient CS (p-value)	Beta-Coefficient IS (p-value)	Beta-Coefficient PAG (p-value)	Beta-Coefficient TMAO (p-value)
Aminoglycosides	1.3e-6 (0.90)	4.0e-4 (0.093)	-1.5e-4 (0.76)	-5.4e-4 (0.25)
Bacitracin	-4.6e-7 (0.61)	-4.6e-6 (0.81)	2.0e-5 (0.64)	-3.6e-5 (0.38)
Betalactams	8.7e-6 (0.50)	2.2e-4 (0.45)	-9.2e-5 (0.88)	-5.0e-4 (0.40)
Cationic Antimicrobial Peptides	-7.9e-7 (0.65)	-1.2e-5 (0.76)	4.0e-5 (0.63)	-6.0e-5 (0.44)
Fluoroquinolones	1.2e-8 (0.88)	2.4e-6 (0.16)	-9.9e-6 (0.01)	5.1e-6 (0.16)
Fosfomycin	1.8e-8 (0.88)	-1.1e-6 (0.69)	-6.3e-7 (0.91)	2.4e-7 (0.96)

Glycopeptides	-4.2e-6 (0.61)	2.6e-4 (0.17)	-5.8e-5 (0.88)	-3.3e-4 (0.39)
Metronizadole	-7.4e-8 (0.52)	6.8e-7 (0.79)	-3.9e-6 (0.48)	1.0e-6 (0.85)
MLS	2.1e-5 (0.39)	1.1e-4 (0.84)	1.0e-5 (0.99)	-4.4e-4 (0.69)
Multi-drug Resistance	-7.1e-7 (0.70)	-1.3e-5 (0.74)	5.0e-5 (0.58)	-8.1e-5 (0.34)
Mupirocin	-4.5e-7 (0.13)	5.8e-6 (0.36)	-1.2e-5 (0.37)	-4.7e-6 (0.72)
Nucleosides	-7.7e-7 (0.77)	8.2e-5 (0.16)	-1.8e-5 (0.88)	-1.1e-4 (0.37)
Phenicol	-1.3e-6 (0.45)	-3.9e-5 (0.32)	1.1e-4 (0.19)	3.2e-5 (0.69)
Sulfonamides	-4.5e-7 (0.28)	3.5e-6 (0.70)	-8.2e-7 (0.97)	-2.2e-5 (0.24)
Tetracyclines	1.1e-5 (0.49)	1.3e-4 (0.71)	-2.1e-4 (0.78)	-4.5e-5 (0.95)
Trimethoprim	3.2e-6 (0.36)	4.8e-5 (0.53)	-9.2e-5 (0.58)	-1.1e-4 (0.50)

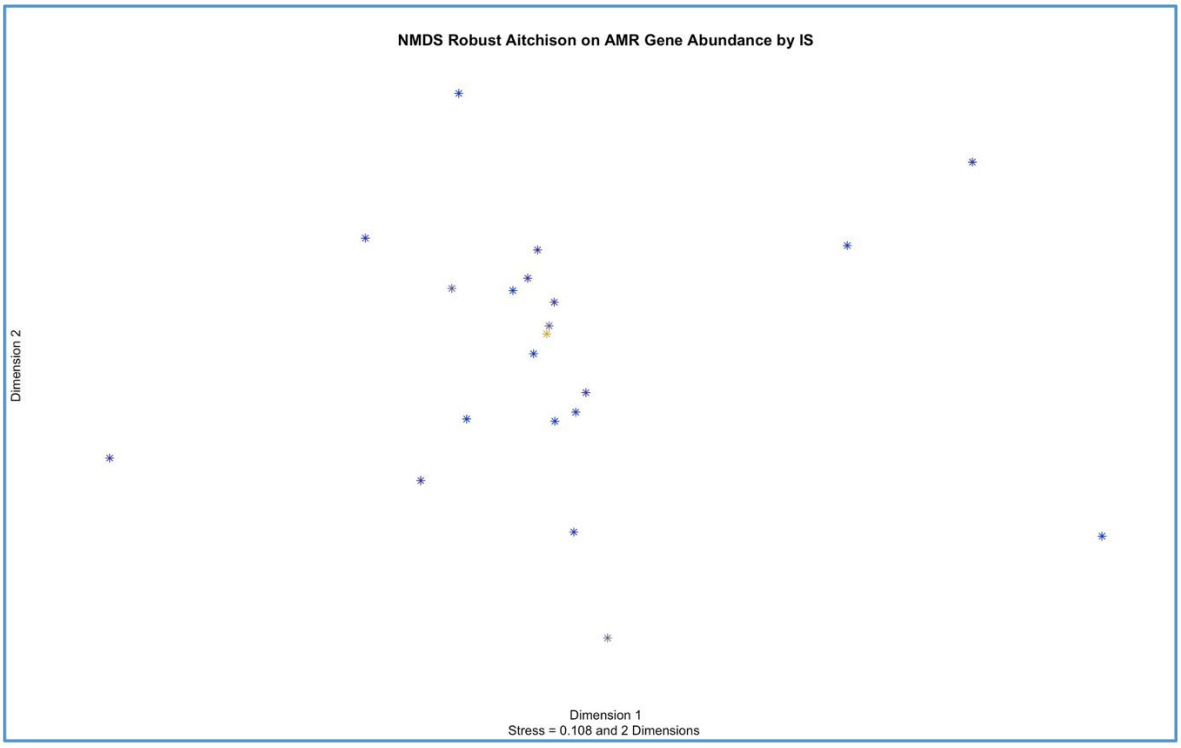
Gene Beta Diversity Assessement by NMDS visualization and PERMANOVA Testing Indicated No Visible or Significant Differences in Beta Diversity by Uremic Toxin Level

For AMR genes, the NMDS function found the most stable solution for ordination to be in a plot of 2 dimensions with a Kruskal Wallis stress calculation of k=0.108. There were no visible differences in beta diversity of AMR genes by uremic toxin level (Figure 1). PERMANOVA confirmed no significant differences based on p-values (CS, p=0.88; IS, p=0.81; PAG, p=0.99; TMAO, p=0.75) summarized in Table 3.

A)


NMDS Robust Aitchison on AMR Gene Abundance by CS
Dimension 2
Dimension 1
Stress = 0.108 and 2 Dimensions

B)


NMDS Robust Aitchison on AMR Gene Abundance by IS
Dimension 2
Dimension 1
Stress = 0.108 and 2 Dimensions

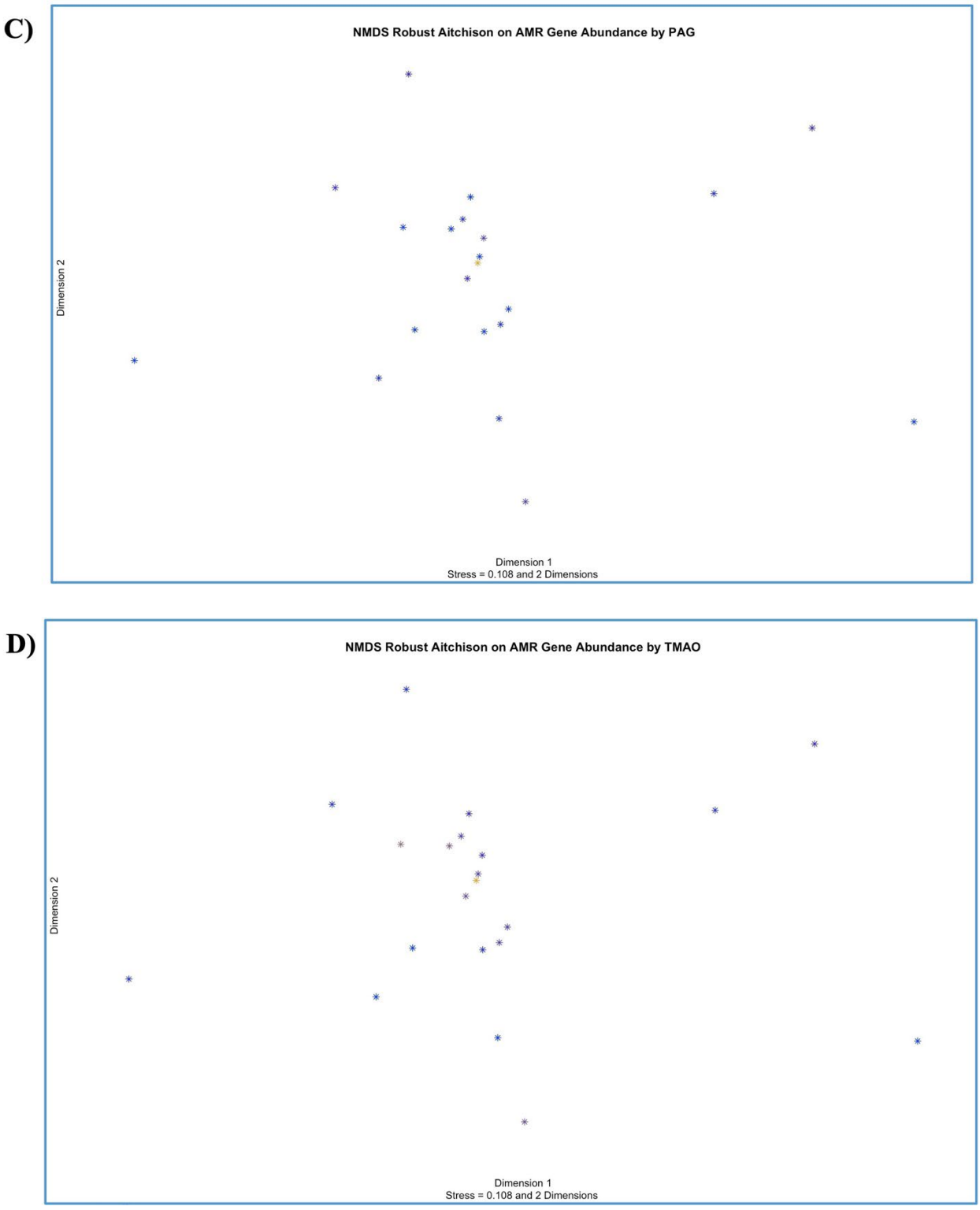


Figure 1. NMDS based on robust Aitchison distance of AMR gene abundance. Points are colored by serum levels of uremic toxins, with dark blue indicating the lowest levels and yellow indicating the highest levels. (A) Plot for *p*-cresyl sulfate (B) Plot for indoxyl sulfate (C) Plot for phenylacetylglutamine (D) Plot for trimethylamine-*N*-oxide.

Table 3. Summary of PERMANOVA Testing Differences in AMR gene beta diversity by uremic toxin molecules

	SUM OF SQUARES	R^2	F	PR(>F)
CS	63.5	0.04	0.64	0.88
IS	63.8	0.04	0.65	0.81
PAG	46.5	0.03	0.47	0.99
TMAO	72.5	0.04	0.84	0.60
RESIDUAL	1481.3	0.82		
TOTAL	1810.9	1.00		

Discussion

This study aimed to explore if gut microbiota carriage of antimicrobial resistance genes was associated with higher levels of uremic toxins. Overall, there were no significant associations between AMR gene abundance and uremic toxin levels, abundance of drug classes of AMR genes and uremic toxin levels, or beta diversity of AMR genes and uremic toxin levels.

Sample size is a major limitation of this study. With a sample size of 21, the multivariate multiple linear regression analysis with 306 AMR genes as outcome variables and 4 predictor variables introduced an increased likelihood of significant results not being truly significant to the sampled population. Indeed, although the results of the multivariate multiple linear regression testing total abundance of AMR genes vs uremic toxin levels initially included 34 AMR genes appearing significant with one of the uremic toxins, after Bonferroni adjustment, no AMR genes had a p-value considered significant. Several of the genes appearing to be significant before Bonferroni adjustment had negative beta-coefficients, suggesting that an increase of the associated uremic toxin molecule would be correlated with a decrease in the AMR gene. Repeating this study's analysis with a larger sample size may help elucidate if any AMR genes carried in the gut have any association with uremic toxins in CKD patients, including a potential negative correlation. However, given the weak correlations seen in the above results, resources may be better utilized exploring other approaches to studying antimicrobial resistance carriage as even.

The null findings of this study suggest that severity of CKD, indicated by uremic toxin levels, is not directly associated with antimicrobial resistance gene carriage in the gut. Although CKD is associated with higher frequency of infection and may increase chances of an AMR infection, this project suggests that increased levels of uremic toxins do not appear to be a direct reason for increased AMR carriage in CKD patients. Instead, AMR infection in CKD patients is likely explained by reasons already known, such as acquiring AMR genes in clinical settings and increased usage of antimicrobial drugs (Pop-Vicas et al., 2008). For clinicians, targeting the factors leading to increased antimicrobial resistance gene carriage in the general population is the best method for targeting this increase in individuals with CKD, as well.

Mechanisms of antimicrobial pollution and increase in antimicrobial resistant microbes in the environment include through the urine and feces of humans and animals given antimicrobials, improper disposal of unused antimicrobials, antimicrobial use in aquaculture and agriculture, and waste from antimicrobial manufacturing (Larsson & Flach, 2022). The AMR genes are then transferred through horizontal gene transmission to other microbes, between humans via direct transmission of microbes, to humans directly from animals via microbe transmission, or from microbes picking up resistance genes directly from the environment (Larsson & Flach, 2022). Suggested methods to control this global spread of AMR genes include prudent antimicrobial prescribing and frequent updating on guidelines for proper antibiotic prescribing that take into account the source of specific infections, such as hospital-acquired or community-acquired, and then for physicians to reference the guidelines before deciding on antimicrobials to prescribe (Lee et al., 2013). Improved compliance to personal hygiene recommendations, particularly for healthcare workers and compliance to hospital sanitation recommendations is recommended to reduce the spread of antimicrobial resistant nosocomial infections (Lee et al., 2013). Additionally, there are recommendations to create new antibiotics or new treatments for microbial infections using antimicrobial peptides, bacteriophages, vaccines, and immunoglobulins (Lloyd, 2012). Reduction in antibiotic use in livestock and aquaculture is also recommended to reduce the intake of AMR genes through food animals (Lee et al., 2013). The National Action Plan for Combating Antibiotic-Resistant Bacteria is the United States government's official strategy to reduce antimicrobial resistance which includes incorporating these strategies (Administration, 2015).

Although patients with CKD experience higher levels of AMR infections, the lack of association between gut AMR gene carriage and uremic toxin levels suggest that an increase in uremic toxins does not specifically impact risk of AMR carriage. To address reducing the risk of AMR infections in the CKD population, strategies to reduce risk of AMR infections for the general population must continue to be developed and executed.

Acknowledgements

This work was supported by the University Scholars Program (USP).

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