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Identification of Antibiotic Administration as a Potentially Novel Factor Associated With Tacrolimus Trough Variability in Kidney Transplant Recipients: A Preliminary Study

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Background. Tacrolimus trough variability is an important risk factor for kidney allograft outcomes. Recent evidence suggests that the gut microbiota is associated with tacrolimus dosing requirements and direct metabolism of tacrolimus. We hypothesize that administration of antibiotics, which are known to alter the gut microbiota, is associated with tacrolimus trough variability. **Methods.** We conducted a retrospective chart review of subjects who received kidney transplants at our institution from 2012 to 2013 and evaluated subjects who received antibiotics during the first month of transplantation (Abx Group, N = 60) and subjects who did not (No Abx Group, N = 200). We evaluated whether antibiotic administration in the Abx Group had increased tacrolimus trough concentrations and concentration over tacrolimus dosage (C/D) after antibiotic administration. We also evaluated tacrolimus variability as measured by standard deviation (SD) and coefficient of variation between the Abx Group and No Abx Group. **Results.** In the Abx Group, tacrolimus trough concentration over tacrolimus dosage (C/D) increased 7 and 15 days after antibiotic administration ($P = 0.001$, $P = 0.07$, respectively, Wilcoxon signed-rank test). From postoperative day 31–45, the variability in tacrolimus trough levels in the Abx Group as measured by SD and coefficient of variation was significantly higher than the variability in the No Abx Group ($P = 0.03$, $P = 0.02$, Wilcoxon rank sum test, respectively). **Conclusions.** Our identification of antibiotic administration as a potentially new risk factor for tacrolimus trough variability suggests the need to carefully follow tacrolimus trough levels after antibiotic administration.

(*Transplantation Direct* 2019;5: e485; doi: 10.1097/TXD.0000000000000930. Published online 23 August, 2019.)

Tacrolimus is one of the most widely used and effective immunosuppressive agents in solid organ transplantation. It is known to have a narrow therapeutic index with supratherapeutic tacrolimus trough levels being associated with nephrotoxicity and neurotoxicity and subtherapeutic trough levels being associated with acute rejection.[1] Many factors, such as age and CYP3A4/CYP3A5 polymorphisms, have been associated with inter-patient tacrolimus dosing requirements.[2-4] They, however, do not fully account for intra-patient tacrolimus trough variability.

Existing data suggest that tacrolimus trough variability is a clinically significant risk factor for kidney allograft outcomes. Sapir-Pichhadze et al[5] conducted a retrospective cohort chart review of 356 adult kidney transplant recipients and reported that an increase in the variability in tacrolimus trough levels was associated with an increased risk for the composite endpoint of late acute rejection, transplant

Received 1 June 2019. Revision received 18 July 2019.

Accepted 19 July 2019.

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Y.Z. and J.R.L. participated in the research design, collection of data, data analysis, and writing of the article. A.M. and M.W. participated in the collection of data, data analysis, and writing of the article. S.K., D.D., and M.L. participated in data analysis and writing of the article.

J.R.L. receives research support from BioFire Diagnostics, L.L.C. The other authors declare no conflicts of interest.

This work was supported, in part, by a K23 AI 124464 from the National Institute of Allergy and Infectious Diseases to J.R.L.

Supplemental digital content (SDC) is available for this article. Direct URL citations appear in the printed text, and links to the digital files are provided in the HTML text of this article on the journal's Web site (www.transplantationdirectjournal.com).

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ISSN: 2373-8731

DOI: 10.1097/TXD.0000000000000930

glomerulopathy, graft failure, or death. Borra et al[6] conducted a retrospective cohort of 297 kidney transplant recipients and reported that high intra-individual tacrolimus trough variability is a risk factor associated with the composite endpoint of allograft loss, biopsy proven chronic allograft nephropathy, or doubling of plasma creatinine. Other studies report similar findings with the use of cyclosporine maintenance immunosuppression.[7,8] Currently known factors associated with intra-patient tacrolimus trough variability are drug-drug interactions that influence CYP3A4 and CYP3A5 metabolism,[1] but they do not fully account for intra-patient tacrolimus variability.

Recent evidence suggests that the gut microbiota can influence absorption, metabolism, and disposition of drugs. In a pilot study of kidney transplant recipients, fecal *Faecalibacterium prausnitzii* abundance early after transplantation was positively associated with future tacrolimus dosing at 1 month.[9] Based upon this work, Guo et al[10] investigated the ability of *F. prausnitzii* to metabolize tacrolimus and reported that *F. prausnitzii* and over 20 other commensal bacterial taxa directly metabolize tacrolimus in vitro into a less-effective immunosuppressive metabolite.[10] Based on these 2 studies, we formulated and tested the hypothesis that antibiotic administration in kidney graft recipients increases tacrolimus trough variability. While some antibiotics, like erythromycin and rifampin, are known to competitively inhibit or induce CYP3A4 or CYP3A5 metabolism, it is unknown whether antibiotics metabolized primarily through other pathways, including those in the penicillin and cephalosporin class, predictably affect tacrolimus trough concentrations. To our knowledge, there are no studies which systematically analyzed the class effect of antibiotics on tacrolimus trough levels and variability.

In this retrospective chart review, we investigated whether antibiotic administration was associated with increased tacrolimus trough variability in the first 45 days after kidney transplantation in 280 kidney transplant recipients.

MATERIALS AND METHODS

Study Population

We conducted a retrospective study of 280 consecutive adult kidney transplant recipients who received their transplantation at New York Presbyterian Hospital, Weill Cornell Medical Center, from January 2012 to June 2013. We focused on the first 45 days of transplantation as tacrolimus trough levels were measured frequently, at least once a week, during this early time after transplantation. Demographic information, antibiotic administration, and tacrolimus troughs and doses were reviewed using electronic medical records. The Weill Cornell Institutional Review Board approved this retrospective study.

Antibiotic Grouping

Among the 280 kidney transplant recipients, 20 were excluded for either death, allograft loss, or switch from tacrolimus to another immunosuppressive agent or were lost to follow-up before postoperation (postop) day 45. The subjects were grouped into whether they received antibiotics during the first 30 days of transplantation besides *Pneumocystis jiroveci* prophylaxis and preoperative surgical prophylaxis (ABX Group, N = 60) or whether they did not receive antibiotics during the first 30 days of transplantation (N = 200) (Figure 1).

Immunosuppressive and Prophylaxis Regimen

Induction immunosuppression included anti-thymocyte globulin therapy or basiliximab therapy. Maintenance immunosuppression consisted of oral tacrolimus and mycophenolate mofetil and in some cases with maintenance steroids based on immunological risk. Tacrolimus was initiated at 2 mg oral twice daily. Tacrolimus trough levels were measured at least weekly and transplant physicians adjusted tacrolimus dosing with a goal tacrolimus trough level of 8–10 ng/mL in the first 3 months of transplantation.

Standard preoperative antibiotic prophylaxis consisted of 1 dose of cefazolin. Standard *P. jiroveci* prophylaxis included

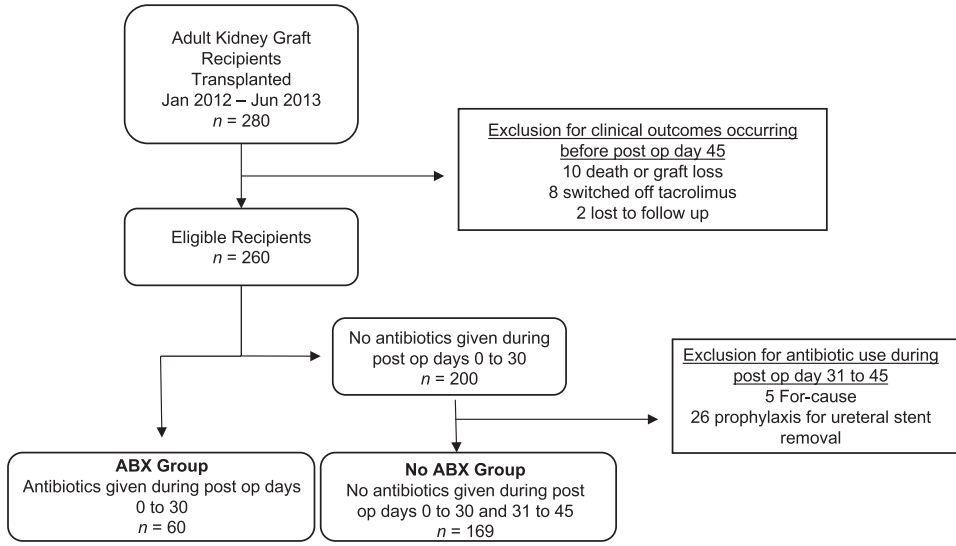

FIGURE 1. Study cohort. From January 2012 to June 2013, 280 adult subjects received kidney transplants. Twenty subjects who either died, lost their allograft, switched off tacrolimus, were lost to follow-up by postop day 45, or did not have tacrolimus trough levels between postop days 31–45 were excluded, leaving 260 subjects eligible for study. Subjects were divided into 2 groups: subjects who received antibiotics during the first 30 days of transplantation (ABX Group, n = 60) and subjects who did not receive antibiotics during the first 30 days of transplantation (n = 200). Among the 200 subjects who did not receive antibiotics during the first 30 days of transplantation, we also excluded 31 subjects who received antibiotics during postop days 31–45 (No ABX Group, n = 169).

trimethoprim-sulfamethoxazole (TMP-SMX) 400–80 mg daily, and standard Cytomegalovirus prophylaxis included acyclovir for 3 months or valgancyclovir for 6 or 9 months depending on cytomegalovirus donor/recipient status. Importantly, clotrimazole, an inhibitor of CYP3A4 and tacrolimus metabolism, was given to each subject as 10 mg twice daily. Clotrimazole was decreased to 10 mg daily at approximately postop day 45 and stopped at approximately postop day 90 (3 mo posttransplantation).

From the electronic medical records, we extracted tacrolimus trough levels during postop day 0 to postop day 45. Tacrolimus trough levels were measured at the New York Presbyterian Hospital, Weill Cornell Medical Center, using ultra high performance liquid chromatography followed by tandem mass spectrometry on a Waters TQD System using the Waters MassTrek Immunosuppressant Kit (Waters, Milford, MA).[11] Tacrolimus trough levels that resulted in ≤2 or ≥15 ng/mL were individually reviewed for verification of 12 hour trough and excluded if the subject had not received at least 3 doses of tacrolimus or if the subject had taken tacrolimus just before blood drawing. Tacrolimus trough levels and clotrimazole dosing, corresponding to postop days of interest, were assigned based on the tacrolimus trough levels and clotrimazole dosing on that exact day or the next available tacrolimus trough levels within 15 days.

Study Outcomes and Statistical Analysis

Demographic and transplantation characteristics were reviewed and compared between the ABX group and No ABX group using the Wilcoxon rank sum test for continuous variables and Fisher exact test for categorical variables.

Within the ABX group, we evaluated whether antibiotics affected tacrolimus dose, tacrolimus trough levels, and tacrolimus concentration-to-dose ratios (C/D, units ng/mL:mg/day) from preantibiotic (pre-Abx) day to postantibiotic (post-Abx) day 7 and post-Abx day 15. Pre-Abx day was defined as the day of or immediately preceding the first antibiotic use during which a tacrolimus trough level was measured. Postantibiotic tacrolimus trough levels and doses were obtained from the clinical encounter on or immediately following the represented post-Abx day. Wilcoxon signed-rank tests were utilized to compare Pre-Abx values with Post-Abx day 7 values and compare Pre-Abx values with Post-Abx day 15 values. Subgroup analyses were performed for the most commonly used antibiotics: penicillin class, cephalosporin class, and fluoroquinolone class.

To evaluate the relationship between antibiotics and tacrolimus trough level variability, we quantified tacrolimus trough level variability by calculating intra-patient standard deviation (SD) ($SD = \sqrt{\sum_{i=1}^{N} (Tacro_i - Tacro_{mean})^2 / (N-1)}$ where N represents number of observations and Tacro represents tacrolimus trough level) and coefficient of variation (CV) ($CV = SD/Tacro_{mean}$) during postop days 31–45. We compared the SD and CV between the ABX group and No ABX group using Wilcoxon rank sum tests. Univariate linear regression was utilized for characteristics associated with both SD and CV and a multivariable linear regression was utilized for characteristics with univariate $P < 0.10$.

All statistical analyses were performed in R 3.3.3 in RStudio 1.1.463.

RESULTS

Kidney Transplant Cohort Characteristics

Among the 280 adult recipients of kidney allograft, 20 were excluded because they (1) died or lost their allograft within the first 45 days of transplantation (N = 10); (2) changed immunosuppression from tacrolimus to another agent within the first 45 days of transplantation (N = 8); or (3) were lost to follow-up (N = 2) before postop day 45. The remaining 260 subjects were divided into whether they received antibiotics during the first 30 days of transplantation besides preoperative surgical prophylaxis and *P. jiroveci* prophylaxis (ABX group, N = 60) or whether they did not receive antibiotics during the first 30 days of transplantation (N = 200). Of the 200 subjects who did not receive antibiotics in the first 30 days of transplantation, we excluded 31 subjects who received antibiotics during postop days 31–45, as we wanted to examine the effect of antibiotics between postop day 31 and postop day 45 without interference from ongoing antibiotic administration, leaving 169 subjects (No ABX Group, N = 169) (Figure 1).

The demographic and transplant characteristics for the ABX group and No ABX group are listed in Table 1. The distribution of demographics and transplantation characteristics were similar between the 2 groups except for the organ donor type being deceased donor which was more frequent in the ABX group than the No ABX group (57% vs 36%, $P = 0.01$, Fisher exact test) (Table 1). In the ABX Group, antibiotics of the fluoroquinolone class (levofloxacin and ciprofloxacin) was the most commonly used antibiotic class followed by the

TABLE 1.

Demographics and transplant characteristics

Characteristics	ABX group (N = 60)	No ABX group (N = 169)	P[a]
Age (y), median	59	56	0.18
Black race, n (%)	11 (18)	23 (14)	0.40
Female, n (%)	25 (42)	54 (32)	0.21
Weight (kg), median	78.4	78.6	0.87
Deceased donor transplantation, n (%)	34 (57)	61 (36)	**0.01**
History of diabetes mellitus, n (%)	15 (25)	50 (30)	0.62
Thymoglobulin induction, n (%)	54 (90)	154 (91)	0.80
Steroid maintenance, n (%)	16 (27)	38 (22)	0.60
PCP TMP-SMX[b] prophylaxis, n (%)	56 (93)	161 (95)	0.52

[a]Continuous variables were compared using Wilcoxon rank sum test. Categorical variables were compared using Fisher exact test. *P* values < 0.05 are in bold.
[b]*Pneumocystis jiroveci* trimethoprim-sulfamethoxazole prophylaxis.

TABLE 2.

Antibiotic use by class or by specific antibiotic during the first mo of transplantation

Antibiotic class or name	ABX Group (n = 60); n, subjects
Fluoroquinolone	25
Penicillin	26
Cephalosporin	19
Vancomycin intravenous	7
Vancomycin oral	4
Nitrofurantoin	3
Linezolid	2
Metronidazole	2
Azithromycin	1
Clindamycin	1
Doxycycline	1
SMX-TMP (for cause)	1

As some subjects received multiple antibiotics, the total number of antibiotics administered exceeds the number of subjects.
SMX-TMP, sulfamethoxazole-trimethoprim.

penicillin class (including pipericillin-tazobactam, amoxicillin, and ampicillin) and the cephalosporin class (including cefazolin, cephalexin, cefpodoxime, and ceftriaxone) (Table 2).

Increased Tacrolimus Variability as Measured by Concentration Over Dosage After Antibiotic Administration

Among the 60 subjects who received antibiotics in the first 30 days of transplantation, 48 subjects had at least 1 tacrolimus trough measured before antibiotic administration, allowing us to study post antibiotic changes at post-Abx day 7 and post-Abx day 15. We evaluated whether antibiotic administration was associated with changes in tacrolimus dose, tacrolimus trough concentrations, or concentration-to-dose ratios (C/D, units ng/mL:mg/day) on post-Abx day 7 and post-Abx day 15 when compared with pre-Abx day.

We found that tacrolimus dose was not significantly different between the median pre-Abx day dose of 4 mg/day and median post-Abx day 7 dose of 4 mg/day (P = 0.17, Wilcoxon signed-rank test) or between the median pre-Abx day dose of 4 mg/day to the median post-Abx day 15 dose of 4 mg/day (P = 0.82) (Figure 2A). However, we observed that the tacrolimus trough level increased from the median pre-Abx day level of 8.2 ng/mL to the median post-Abx day 7 level of 9.0 ng/mL (P = 0.08) and from the median pre-Abx day level of 8.2 ng/mL to the median post-Abx day 15 level of 8.9 ng/mL (P = 0.06) (Figure 2B) (Table 3). Tacrolimus C/D significantly increased from the median pre-Abx ratio of 2.0 to the median post-Abx day 7 ratio of 2.4 (P = 0.001) and from the median pre-Abx ratio of 2.0 to the median post-Abx day 15 ratio of 2.0 (P = 0.07) (Figure 2C) (Table 4).

Because diarrhea is associated with elevated tacrolimus trough levels,[12] we reviewed whether the 48 subjects developed diarrhea after antibiotic usage. Among the cohort, 25 subjects developed diarrhea within the 15 days after antibiotic use (Diarrhea Cohort) and 23 subjects did not (No Diarrhea Cohort). Within the Diarrhea Cohort, there was a trend towards significance for the tacrolimus trough concentration from the median pre-Abx level of 8 ng/mL to the median post-Abx day 15 level of 9.5 ng/mL (P = 0.098) and a trend towards significance for the tacrolimus C/D from the median pre-Abx ratio of 2.0 to the median post-Abx day 7 ratio of 2.4 (P = 0.057) (**Figure S1, SDC**, http://links.lww.com/TXD/A221) (Tables 3 and 4). Interestingly, within the No Diarrhea Cohort, the tacrolimus C/D significantly increased from the median pre-Abx ratio of 1.9 to the median post-Abx day 7 ratio of 2.4 (P = 0.01) (**Figure S2, SDC**, http://links.lww.com/TXD/A221) (Tables 3 and 4).

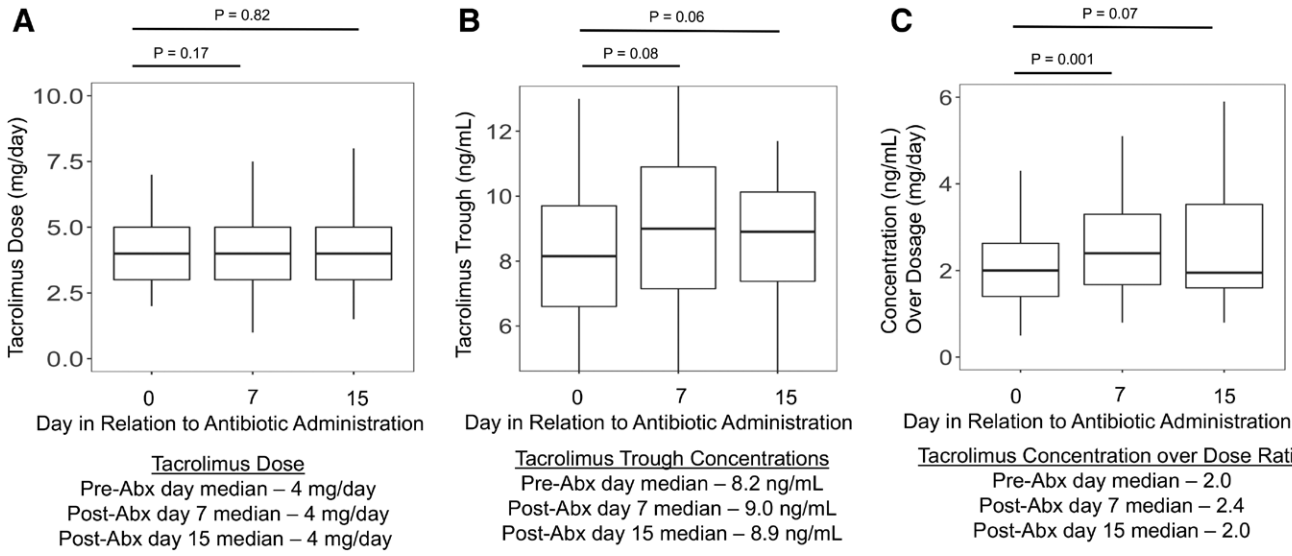

FIGURE 2. Tacrolimus dose, trough levels, and concentration-to-dose ratio after antibiotic administration. Box-and-Whisker plots with day on the *x*-axis and (A) tacrolimus dose, (B) tacrolimus trough, or (C) tacrolimus concentration-to-dose ratios on the *y*-axis in the 48 subjects who received antibiotics in the first mo of transplantation and had tacrolimus trough before the first antibiotic exposure. In each Box-and-Whisker plot, the horizontal line within each box represents the median, the bottom and top of each box represents the 25th and 75th percentile values, the top whisker represents the 75th percentile value plus 1.5 times the interquartile range, and the bottom whisker represents the 25th percentile value minus 1.5 times the interquartile range. Pre-Abx day refers to the day on or immediately before the first antibiotic exposure. Values below each plot for pre-Abx day, post-Abx day 7, and post-Abx day 15 are presented as medians. Statistical comparisons were made using Wilcoxon signed-rank test.

TABLE 3.

Changes in tacrolimus trough over time after antibiotic administration in the different cohorts

		Pre-Abx	Post-Abx day 7	Post-Abx day 15
Group	**Number of patients**	**Median**	**Median (*P*)**	**Median (*P*)**
All ABX patients	48	8.2	9.0 (0.08)	8.9 (0.06)
Diarrhea cohort	25	8.0	9.2 (0.19)	9.5 (0.098)
No diarrhea cohort	23	8.2	8.7 (0.24)	8.4 (0.35)
Steroid Maintenance group	11	7.9	8.3 (0.76)	9.5 (0.41)
Steroid Free group	37	8.2	9.3 (0.08)	8.8 (0.11)

All median values are tacrolimus troughs (ng/mL). The *P* value is a Wilcoxon signed-rank test, comparing the respective column to the Pre-Abx Group.

TABLE 4.

Changes in tacrolimus concentration over dosage over time after antibiotic administration in the different cohorts

		Pre-Abx	Post-Abx day 7	Post-Abx day 15
Group	**Number of patients**	**Median**	**Median (*P*)**	**Median (*P*)**
All ABX patients	48	2.0	2.4 (0.001)	2.0 (0.07)
Diarrhea cohort	25	2.0	2.4 (0.057)	2.0 (0.16)
No diarrhea cohort	23	1.9	2.4 (0.01)	1.9 (0.23)
Steroid Maintenance group	11	2.1	2.3 (0.29)	2.0 (0.33)
Steroid Free group	37	1.9	2.4 (0.003)	1.9 (0.14)

All median values are tacrolimus concentration over dose. The *P* value is a Wilcoxon signed-rank test, comparing the respective column to the Pre-Abx Group.

We also evaluated whether steroid maintenance was associated with elevated tacrolimus trough levels. Among the cohort, 11 subjects were under a steroid maintenance protocol (Steroid Maintenance Group) and 37 subjects were under a steroid-free protocol (Steroid Free Group). Within the Steroid Maintenance Group, there were no significant differences with respect to tacrolimus dosage, tacrolimus trough levels, or tacrolimus C/D among the different time frames (**Figure S3, SDC**, http://links.lww.com/TXD/A221) (Tables 3 and 4). Within the Steroid Free Group, there was a trend towards significance for the tacrolimus trough level from the median pre-Abx level of 8.2 ng/mL to the median post-Abx day 7 level of 9.3 ($P = 0.08$), and there was a significant increase in the tacrolimus C/D from the median pre-Abx ratio of 1.9 to the median post-Abx day 7 ratio of 2.4 ($P = 0.003$) (**Figure S4, SDC**, http://links.lww.com/TXD/A221) (Tables 3 and 4).

We also evaluated whether the type of antibiotic used was associated with altered tacrolimus pharmacokinetics after administration in the above cohort of 48 subjects analyzed at the time of first antibiotic use. For each class of antibiotics, we included subjects if they received the antibiotic class during the first month after transplantation and within a day of the Pre-Abx date. The ABX subgroup that received penicillin class antibiotics (N = 16) had an increase in tacrolimus trough level from pre-Abx to post-Abx day 15 ($P = 0.07$) and an increase in tacrolimus C/D from pre-Abx to post-Abx day 7 ($P = 0.096$) and from pre-Abx to post-Abx day 15 ($P = 0.04$) (Figure 3A, 3D). The ABX subgroup that received cephalosporin class antibiotics (N = 8) had an increase in tacrolimus trough level from pre-Abx to post-Abx day 7 ($P = 0.04$) and an increase in tacrolimus C/D from pre-Abx to post-Abx day 7 ($P = 0.055$) (Figure 3B, 3E). The ABX subgroup that received fluoroquinolone class antibiotics (N = 18) did not have increased tacrolimus trough levels or C/D from pre-Abx to post-Abx day 7 or from pre-Abx to post-Abx day 15 (Figure 3C, 3F).

Antibiotics in the First Month of Transplantation Increase Tacrolimus Trough SD and Coefficent of Variation

Based on our observation that antibiotics increase tacrolimus trough levels and tacrolimus concentration-to-dose ratios, it is plausible that antibiotics could also increase tacrolimus trough level variability. We calculated the median intra-patient SD of tacrolimus trough levels during postop days 31–45. We were unable to calculate these SDs in 6 patients in the ABX Group and 38 patients in the No ABX Group because there were only 1 or 0 tacrolimus levels during this time period (Table 5). Intra-patient SD of tacrolimus trough levels was significantly higher in the ABX group than the No ABX group (median 2.6 vs 1.6, $P = 0.03$, Wilcoxon rank sum test) (Figure 4A). The median intra-patient CV of tacrolimus trough levels during postop days 31–45 was also significantly higher in the ABX group compared with the No ABX group (median 0.29 vs 0.18, $P = 0.02$, Wilcoxon rank sum test) (Figure 4B). There were, however, no differences in the tacrolimus trough levels between the 2 groups (median 8.9 vs 8.9 ng/mL, respectively, $P = 0.88$, Wilcoxon rank sum test). The number of measurements was significantly higher in in the ABX group than the No ABX group (median 2 vs 2, respectively, $P = 0.002$, Wilcoxon rank sum test) (Table 5).

A linear regression for the SD was performed using the following characteristics: age, weight, female gender, history of diabetes mellitus, steroid maintenance, discontinuation of clotrimazole by postop day 45, diarrhea during postop day 31 to post opday 45, and antibiotic group status. In univariate analysis, steroid maintenance, clotrimazole discontinuation, and diarrhea during time frame were associated with SD ($P < 0.10$), and in multivariate analysis, steroid maintenance and clotrimazole discontinuation continued to be associated with SD ($P < 0.10$) (Table 6).

A linear regression for the CV was performed using the following characteristics: age, weight, female gender, history

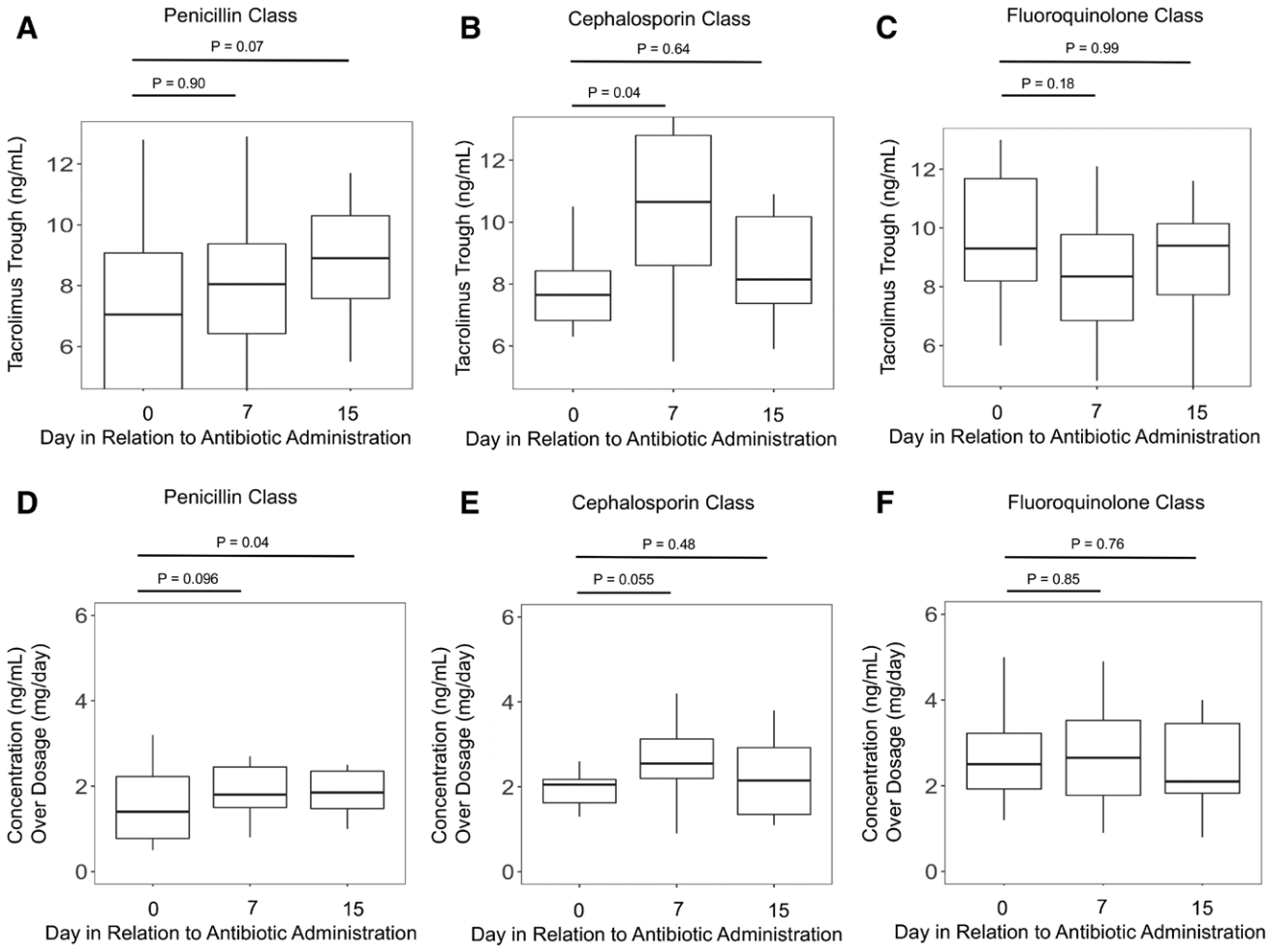

FIGURE 3. Tacrolimus trough levels and concentration-to-dose ratios by antibiotic class. A, Box-and-Whisker plot with the day in relation to antibiotic administration on the *x*-axis and the tacrolimus trough levels on the *y*-axis for the 16 subjects who received a penicillin class antibiotic. The *P* values were calculated using Wilcoxon signed-rank test. B, Box-and-Whisker plot with the day in relation to antibiotic administration on the *x*-axis and the tacrolimus trough levels on the *y*-axis for the 8 subjects who received a cephalosporin class antibiotic. The *P* values were calculated using Wilcoxon signed-rank test. C, Box-and-Whisker plot with the day in relation to antibiotic administration on the *x*-axis and the tacrolimus trough levels on the *y*-axis for the 18 subjects who received a fluoroquinolone class antibiotic. The *P* values were calculated using Wilcoxon signed-rank test. D, Box-and-Whisker plot with the day in relation to antibiotic administration on the *x*-axis and the concentration-to-dose ratios on the *y*-axis for the 16 subjects who received a penicillin class antibiotic. The *P* values were calculated using Wilcoxon signed-rank test. E, Box-and-Whisker plot with the day in relation to antibiotic administration on the *x*-axis and the concentration-to-dose ratios on the *y*-axis for the 8 subjects who received a cephalosporin class antibiotic. The *P* values were calculated using Wilcoxon signed-rank test. F, Box-and-Whisker plot with the day in relation to antibiotic administration on the *x*-axis and the concentration-to-dose ratios on the *y*-axis for the 18 subjects who received a fluoroquinolone class antibiotic. The *P* values were calculated using Wilcoxon signed-rank test.

of diabetes mellitus, steroid maintenance, discontinuation of clotrimazole by postop day 45, diarrhea during postop day 31 to postop day 45, and antibiotic group status. In univariate analysis, steroid maintenance, clotrimazole discontinuation, diarrhea during time frame, and ABX status were associated with CV ($P < 0.10$) and in multivariate analysis, steroid maintenance and clotrimazole discontinuation continued to be associated with CV ($P < 0.10$) (Table 7).

DISCUSSION

In this study, we report that antibiotics increase tacrolimus trough variability as measured by C/D. To the best of our knowledge, this is the first study that systematically examined tacrolimus trough levels after antibiotic administration. Prior evidence exists mostly in case report and case report series. In 1 case, ceftriaxone was reported to be associated with doubling of cyclosporine levels, either alone or in combination with other antibiotics (azithromycin, gentamicin) and resolved with discontinuation of antibiotics.[13] Case reports have also reported azithromycin in increasing cyclosporine trough levels[14] and tacrolimus trough levels[15] and ciprofloxacin in increasing cyclosporine levels.[16] In a pharmacokinetics study of 10 kidney transplant recipients with urinary tract infections, 6 days of levofloxacin administration was shown to result in a 25% increase in the Area Under the Curve for oral cyclosporine microemulsion and oral tacrolimus.[17]

Our identification of antibiotics as a potential new risk factor for tacrolimus trough variability advances the understanding of tacrolimus, a drug that has a narrow therapeutic index with supratherapeutic tacrolimus trough levels being associated with nephrotoxicity and neurotoxicity and with subtherapeutic trough levels being associated with acute rejection. Importantly, diarrhea has been associated with increases

TABLE 5.

Tacrolimus trough level variability between the ABX group and No ABX group

Group	Number of patients	Tacrolimus trough	Tacrolimus SD	Tacrolimus CV	Number of tacrolimus measurements
ABX group	54	8.9	2.6	0.29	2
No ABX group	131	8.9	1.6	0.18	2
P[a]		0.88	**0.03**	**0.02**	**0.002**

Values are presented as medians. SDs of tacrolimus levels during postop days 31–45 were unable to be calculated in 4 patients in the ABX group and 38 patients in the No ABX group because there was only one or no tacrolimus levels during this time period. Comparison of tacrolimus trough levels during postop days 31–45 from the remaining patients showed no differences between the ABX Group and No ABX Group. The ABX Group had significantly higher SD and CV than the No ABX Group. The number of tacrolimus trough level measurements during postop days 31–45 was also higher in the ABX Group compared with the No ABX Group.

[a]*P* value calculated by Wilcoxon rank sum test. *P* values < 0.05 are in bold.

CV, coefficient of variation; SD, standard deviation.

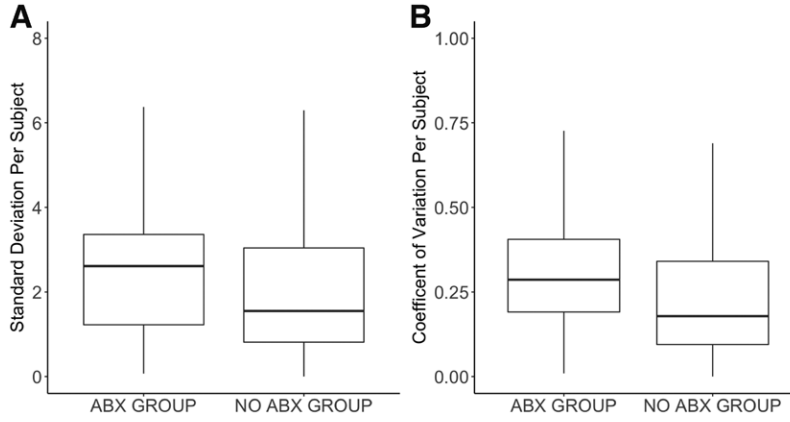

FIGURE 4. Increased tacrolimus variability in the ABX group compared with the No ABX group. A, Box-and-Whisker plots with antibiotic exposure on the *x*-axis and SD of tacrolimus trough levels during postop days 31 and 45 on the *y*-axis. In each Box-and-Whisker plot, the horizontal line within each box represents the median, the bottom and top of each box represents the 25th and 75th percentile values, the top whisker represents the 75th percentile value plus 1.5 times the interquartile range, and the bottom whisker represents the 25th percentile value minus 1.5 times the interquartile range. ABX group mean SD was 2.6 ng/mL and the No ABX group median SD was 1.6 ng/mL ($P = 0.03$, Wilcoxon rank sum test). B, Box-and-Whisker plots with antibiotic exposure on the *x*-axis and the coefficient of variation of tacrolimus trough levels during postop days 31–45 on the *y*-axis. In each Box-and-Whisker plot, the horizontal line within each box represents the median, the bottom and top of each box represents the 25th and 75th percentile values, the top whisker represents the 75th percentile value plus 1.5 times the interquartile range, and the bottom whisker represents the 25th percentile value minus 1.5 times the interquartile range. ABX group median CV was 0.29 and No ABX group median CV was 0.18 ($P = 0.02$, Wilcoxon rank sum test). CV, coefficient of variation; SD, standard deviation.

TABLE 6.

Linear regression of characteristics associated with tacrolimus SD

	Univariate analysis			Multivariate analysis		
Characteristics	**Estimate**	**SE**	***P***	**Estimate**	**SE**	***P***
Age	0.02	0.01	0.14			
Weight	–0.01	0.01	0.45			
Female gender	0.31	0.28	0.28			
History of diabetes mellitus	0.22	0.29	0.45			
Steroid maintenance	0.56	0.32	0.08	0.55	0.31	0.08
Clotrimazole discontinued status	0.65	0.32	0.05	0.65	0.32	0.04
Diarrhea during time frame	0.56	0.33	0.09	0.48	0.33	0.14
ABX status	0.39	0.30	0.19			

Univariate linear regression was performed with the dependent variable being tacrolimus SD and the characteristics listed in the table as independent variables. The estimate, SE, and *P* Value are listed for each characteristic. Characteristics that were associated with a $P < 0.10$ were included in a multivariate linear regression analysis.

SD, standard deviation.

in tacrolimus trough levels via a P-glycoprotein mechanism.[12] We also evaluated the effect of diarrhea in the setting of antibiotic use and continued to report an increase in tacrolimus C/D after antibiotic use in subjects that did not develop diarrhea, suggesting that antibiotics in the absence of diarrhea may also have an effect on tacrolimus trough variability. Altogether, our data suggest the need to measure tacrolimus trough levels after administration of antibiotics. It is important to note that the tacrolimus trough levels do not increase in all cases. We hypothesize that antibiotics influence gut microbial community structure and composition and contribute to the tacrolimus trough variability. Our hypothesis is based in part on findings that the abundance of fecal *F. prausnitzii* in fecal specimens from kidney graft recipients is associated with

TABLE 7.

Linear regression of characteristics associated with tacrolimus CV

	Univariate analysis			Multivariate analysis		
	Estimate	SE	*P*	Estimate	SE	*P*
Age	0.00	0.00	0.21			
Weight	0.00	0.00	0.30			
Female gender	0.03	0.03	0.37			
History of diabetes mellitus	0.02	0.03	0.6			
Steroid maintenance	0.07	0.03	0.04	0.06	0.03	0.04
Clotrimazole discontinued status	0.11	0.03	<0.001	0.11	0.03	<0.001
Diarrhea during time frame	0.06	0.03	0.06	0.05	0.03	0.13
ABX status	0.05	0.03	0.06	0.04	0.03	0.19

Univariate linear regression was performed with the dependent variable being tacrolimus CV and the characteristics listed in the table as independent variables. The estimate, SE, and *P* Value are listed for each characteristic. Characteristics that were associated with a $P < 0.10$ were included in a multivariate linear regression analysis.
CV, coefficient of variation.

future tacrolimus dosing[9] and commensal bacteria including *F. prausnitzii* can directly metabolize tacrolimus into a less immunosuppressive metabolite.[10] Because there is great interpatient variation of the microbiota in the population, it is possible that the effects of antibiotic administration are not only antibiotic dependent but also gut microbiota dependent. This study, however, did not explore the gut microbiota in relation to antibiotic administration and tacrolimus trough level variability, although research shows that *F. prausnitzii* isolates from healthy volunteers is generally sensitive to the antibiotics analyzed in our study.[18]

In our study, we found that the subgroup of subjects who received penicillin-type antibiotics or cephalosporin-type antibiotics had a trend towards increased C/D after antibiotic administration, while we did not find this association in the subgroup of subjects who received fluroquinolones antibiotics. Our data suggest that the class of antibiotics may be important in determining tacrolimus trough variability. However, the sizes of the subgroups are small and need to be interpreted with caution.

There are several limitations to our study. This is a retrospective study subject to the limitations of retrospective study design. We do not have CYP3A4 and CYP3A5 polymorphisms data on these subjects, which may have influenced tacrolimus trough and dosing in this cohort of subjects. It is possible that antibiotics lead to tacrolimus trough variability via the CYP3A4 or CYP3A5 inhibitor or P-glycoprotein, which could account for some of the variability seen. Some subjects in the ABX Group received multiple antibiotics, and it is difficult to isolate the effect of one particular antibiotic class in such cases. Subjects who did not receive antibiotics in the first 30 days of transplantation but did receive antibiotics from postop days 31–45 were excluded, and this may have introduced selection bias. Given the retrospective nature of the study, we were also unable to obtain the tacrolimus formulation for each patient and whether there were switches to the formulation during the study period, which could also affect variability. Our study also does not answer the question of whether antibiotics have long-term effects on tacrolimus trough variability. In addition, the variability that we have detected in terms of tacrolimus trough levels and C/D is small and may not reflect clinically important variability. Finally and importantly, we did not find a significant association between SD and antibiotic status or between CV and antibiotic status in multivariable analysis, so our data need to be interpreted with caution.

Despite these limitations, we have identified antibiotics as a potentially novel risk factor for increases in tacrolimus trough variability as measured by C/D. Our data require future validation but it does suggest the need for close monitoring of tacrolimus trough levels during and after antibiotic administration. This may decrease the variability in tacrolimus trough fluctuations and thereby mitigate risks that can lead to the complications from supratherapeutic tacrolimus trough levels and subtherapeutic tacrolimus trough levels.

ACKNOWLEDGMENTS

The authors thank Dr. Manikkam Suthanthiran for his overall guidance and editing of the article.

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