OPEN

Application of Various Techniques to Gain Insights Into the Complex Urinary Tract Microbial Communities of Renal Transplant Recipients

Sanchutha Sathiananthamoorthy, PhD,[1] Katia Florman, MBBS,[2] Damien Richard, PhD,[3,4] Ka Kei Cheng, MSc,[1] Vittoria Torri, BSc,[1] Fiona McCaig, MBChB, MD,[2] Mark Harber, MBBS,[2] and Jennifer L. Rohn, PhD[1]

Background. Urinary tract infections (UTIs) are prevalent in renal transplant (RT_X) recipients and associated with worse outcomes. Early detection by sensitive diagnostic tests and appropriate treatment strategies in this cohort is therefore crucial, but evidence has shown that current methods may miss genuine infections. Research has shed light on the urinary tract microbial ecology of healthy individuals and nontransplant patients with UTI, but information on the RTx cohort is scant. We conducted a cross-sectional study to (i) compare the gold standard diagnostic culture with alternative techniques and (ii) characterize RTx patient urinary microbial communities. **Methods.** Midstream urine specimens were collected from 51 RTx patients attending a renal transplant clinic and 27 asymptomatic controls. Urinary microscopy, dipstick, and routine culture were performed. To improve sensitivity of microbial detection, we cultured the urinary cell sediment and performed 16S rRNA gene sequencing on urine. Uroplakin-positive urothelial cells shed in urine were analyzed by immunofluorescence staining for any bacterial association. **Results.** Sediment culture and 16S rRNA sequencing confirmed detection deficiencies of diagnostic culture and revealed differences in the urobiomes of RTx patients and controls. Specifically, *Gardnerella*, *Escherichia*, and *Lactobacillus* were most abundant in patients, whereas *Lactobacillus*, *Streptococcus*, and *Gardnerella* were most abundant in controls. The application of both culture and sequencing provided a more nuanced view of the urinary microbial communities. **Conclusions.** This study provides insight into the potential problems of diagnostic culture within RTx patients and sheds light on their urinary microbial inhabitants. Further work may identify key microbial signatures and facilitate the development of better tools for UTI detection within this cohort, which could allow targeted intervention before an infection leads to serious consequences.
http://links.lww.com/TXD/A479

(*Transplantation Direct* 2023;9: e1418; doi: 10.1097/TXD.0000000000001418).

Received 21 September 2022.

Accepted 4 October 2022.

[1] Department of Renal Medicine, Division of Medicine, Centre for Urological Biology, University College London, Royal Free Hospital Campus, London, United Kingdom.

[2] Renal Unit, Department of Renal Medicine, Division of Medicine, University College London, Royal Free Hospital, London, United Kingdom.

[3] Department of Infection, Immunity and Inflammation, Institute of Child Health, University College London, Great Ormond Street Hospital Campus, London, United Kingdom.

[4] Department of Genetics, Evolution and Environment, Division of Biosciences, UCL Genetics Institute, University College London, London, United Kingdom.

The authors declare no conflicts of interest, aside from J.L.R., who has share options in the University College London, United Kingdom, spinout company AtoCap Ltd, which seeks novel cures for urinary disorders.

S.S. participated in the design, performance of laboratory experiments, data analysis, and write-up. K.F. participated in the data analysis and write-up. D.R. participated in the data analysis and write-up. K.K.C. participated in the performance of laboratory experiments and data analysis. V.T. participated in the performance of laboratory experiments. F.M. participated in the design and patient recruitment. M.H. participated in the design, patient recruitment, and write-up. J.L.R. participated in the design, data analysis, and write-up.

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.transplantationdirect.com).

Correspondence: Sanchutha Sathiananthamoorthy, PhD, Department of Renal Medicine, Division of Medicine, University College London, Royal Free Hospital Campus, Rowland Hill Street, London, NW3 2PF, United Kingdom (sanchutha.sathi@outlook.com); and Jennifer L. Rohn, PhD, Department of Renal Medicine, Division of Medicine, University College London, Royal Free Hospital Campus, Rowland Hill Street, London, NW3 2PF, United Kingdom (j.rohn@ucl.ac.uk).

ISSN: 2373-8731

DOI: 10.1097/TXD.0000000000001418

INTRODUCTION

Approximately 150 million people worldwide develop urinary tract infection (UTI) every year.[1,2] The incidence in renal transplant (RTx) recipients is much higher, with up to 72% of patients reported to experience UTI within the first year of transplantation,[3-6] imposing a substantial financial and clinical burden.[7,8] UTI in RTx patients is linked to several serious consequences, including acute pyelonephritis[9] subsequent allograft dysfunction[10] and loss,[11-13] as well as bloodstream infections,[14-16] sepsis,[17,18] and death.[19,20]

Recurrent UTI (RUTI) is reported in up to 72% of RTx patients, resulting in poorer outcomes and multidrug resistance.[21-23] Early detection is therefore essential, particularly within the posttransplant period of 1–3 mo, when >80% of UTIs occur.[11] Predicting which RTx patients may experience UTI would allow tailored management, improve clinical outcomes, reduce the need for hospitalization, and potentially reduce reliance on prolonged prophylactic antibiotics.

Successful treatment of UTI is hindered by the recognized inadequacies of routine diagnostics.[24-26] Although urinary dipstick has been shown to lack sensitivity,[26,27] we and others have reported that routine midstream urine (MSU) culture misses genuine infection in the presence of symptoms.[24,25,28,29] This technique utilizes 1–10 μL of urine and diagnostic criteria originally applied to acute pyelonephritis patients,[30,31] resulting in the dismissal of $<10^5$ colony-forming units per milliliter (cfu/mL) and mixed growth,[32] both of which may be clinically significant. Despite this, such tests are heavily relied on to confirm infection in symptomatic individuals, including RTx patients.[32] Furthermore, the variation in applied UTI diagnostic criteria between laboratories limits the reliability of epidemiological data.

Matters are complicated by recent evidence that the healthy urinary tract is not sterile but instead inhabited by a diverse and dynamic microbial community—the urobiome.[28,29,33-36] Although its role in symptomatic patients who are otherwise healthy is being clarified, current knowledge of the urobiome in RTx patients is limited.[37-39] Furthermore, the treatment of asymptomatic bacteriuria (ASB) in RTx patients is controversial—indeed, the discovery of the urobiome renders the concept of ASB uncertain.[40-43] Clinical experience has revealed that not all RTx patients respond to treatment in the same way, with evidence suggesting that ASB is often the initial or only manifestation of transplant pyelonephritis and urosepsis.[43,44]

Improved understanding of UTI pathophysiology and the urobiome in RTx patients may inform the development of more sensitive and targeted alternatives to current diagnostic and treatment approaches. We conducted a cross-sectional study with the aims of (i) comparing the microbial compositions of the diagnostic culture with alternative techniques and (ii) characterizing the urinary microbial communities of RTx patients.

MATERIALS AND METHODS

Subject Recruitment and Initial Assessment

Ethical approval was obtained from the National Research Ethics Committee, London, United Kingdom (Ref 05/Q0508/6). Adult male and female RTx patients aged ≥18 y were consented and recruited during a posttransplantation clinical consultation at the Royal Free Hospital Renal Unit, London, United Kingdom. Male and female asymptomatic controls aged ≥18 y, with no known urologic complications or UTI and not on antibiotics in the past 4 wks, were recruited from healthy hospital staff and students. A single MSU was collected from each subject. Once acquired, specimens were analyzed within 10 min by urinary dipstick for inflammatory and infection markers; leukocyte esterase and nitrites (Siemens Healthineers, Germany), and microscopy for leukocytes (WBC) (Olympus, United Kingdom) (Figure 1). Samples were processed promptly to avoid the effect of leukocyte decay with time.

Routine Midstream Urine Culture

MSUs were submitted to the hospital laboratory for MSU culture.[32] Typically, patient samples were kept at room temperature for 15–40 min before being transported to the diagnostic laboratory for MSU culture. Samples that were not processed immediately at the diagnostic laboratory were stored at 4°C. CLED medium (Oxoid, United Kingdom) was inoculated with 2.5 μL of uncentrifuged urine and incubated aerobically at 37°C for 18–24 h. A count of $\geq 10^5$ cfu/mL for 1 organism indicated significant bacteriuria (positive for UTI), whereas titers below threshold were reported as "no significant growth." Cultures with ≥2 organisms were deemed "mixed growth." Because a description of organisms that grew on negative or mixed cultures was not provided, an identical MSU culture was performed on the same specimen in our research laboratory. Samples were stored at 4°C for 1–3 h before subsequent processing in the research laboratory.

Enhanced Urine Sediment Culture and Bacterial Identification

Sediment culture was performed in parallel to enrich for microorganisms, as this was previously shown to be more sensitive.[25] Briefly, 5–15 mL of urine was centrifuged at 1400×*g* for 10 min. The sediment was resuspended in 400 μL of sterile phosphate buffer saline (PBS) (Life Technologies, United Kingdom). Tenfold serial dilutions were performed in PBS, and 50 μL volumes were plated onto ChromID CPS Elite agar (bioMérieux, France). Cultures were incubated aerobically at 37°C for 18–24 h. Following incubation, microbial colonies were identified by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (Bruker Daltonics, United States).

Immunofluorescence Staining

Immunofluorescence staining was performed as described previously.[45] Urine was centrifuged onto glass slides, fixed and stained with wheat germ agglutinin conjugated to Alexa Fluor 488 (Life Technologies, United Kingdom) to identify cellular membranes and anti-uroplakin-IIIa mouse monoclonal antibody (Progen Biotechnik, Germany), followed by goat antimouse antibody conjugated to Alexa Fluor 555 (Life Technologies, United Kingdom) and 4′,6-diamidino-2-phenylindole (Thermo Fisher Scientific, United Kingdom) to stain DNA. Samples were imaged using Leica DM 2500 M Material Analysis Microscope (Leica Microsystems, Germany) with Infinity Capture V6.2.0 software (Teledyne Lumenera, Canada).

16S rRNA Gene Sequencing

Before extraction, 2 mL samples of unspun urine were stored at −80°C. After thawing, samples were pretreated

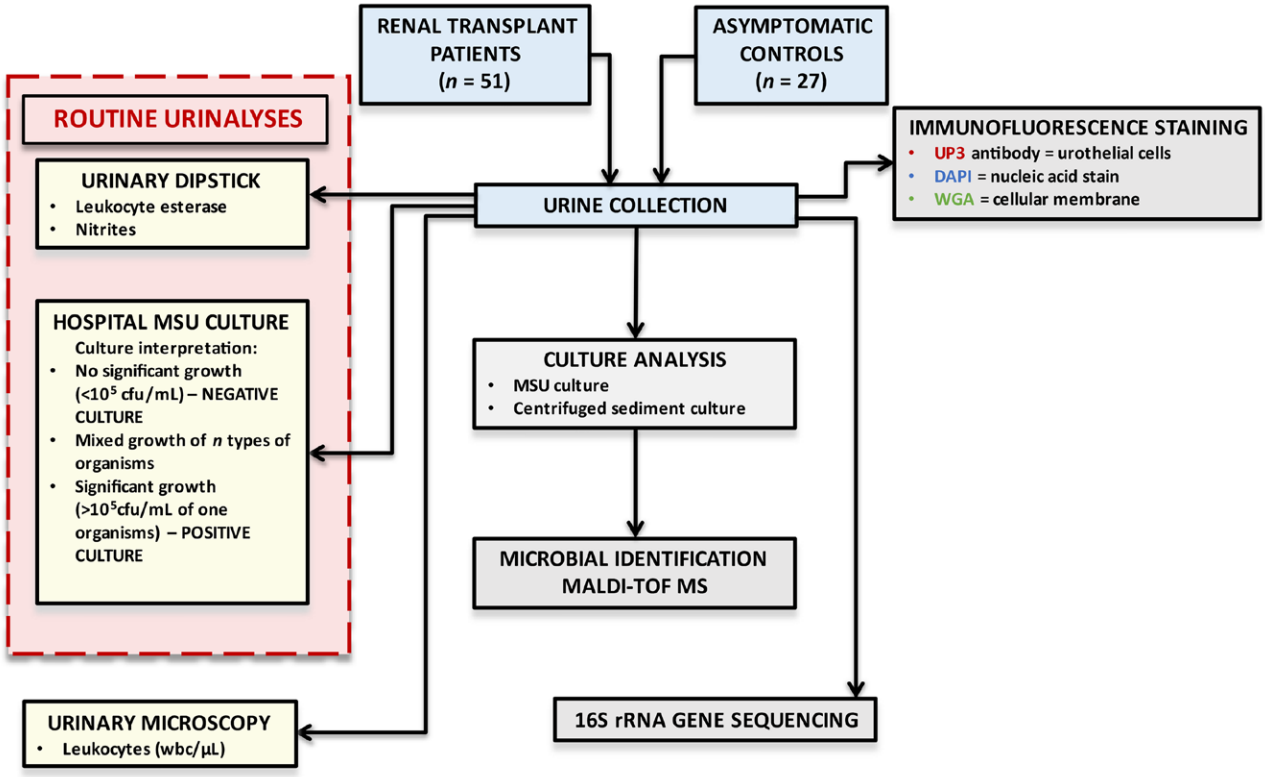

FIGURE 1. Experimental workflow for renal transplant patient and control urine specimens. cfu/mL, colony-forming units per milliliter; DAPI, 4′,6-diamidino-2-phenylindole; MSU, midstream specimen of urine; MALDI-TOF MS, matrix-assisted laser desorption ionization time-of-flight mass spectrometry; rRNA, ribosomal RNA; UP3, uroplakin III; wbc/μL, leukocyte counts per microliter; WGA, wheatgerm agglutinin.

with an enzymatic lysis buffer, consisting of 20 μg/mL of lysozyme, 20 mM Tris-Cl (pH 8.0), 2 mM sodium EDTA, and 1.2% Triton-X-100 for 30 min at 37°C. Genomic DNA was extracted using the DNeasy Blood and Tissue kit (Qiagen Ltd., United Kingdom), following the manufacturer's protocol.[46]

PCR was performed to amplify the V3–V4 hypervariable regions of the 16S rRNA gene using barcoded primers 341F (5′-GGATTAGATACCCBRGTAGTC-3′) and 805R (5′-ACGTCRTCCCCDCCTTCCTC-3′) as described previously.[25] Reactions were performed in duplicate, and DNA concentrations were determined with the Qubit high-sensitivity DNA assay kit and 4.0 fluorometer (Life Technologies, United Kingdom). Amplicons were purified using Agencourt AMPure XP magnetic beads (Beckman Coulter, United States) and combined in equimolar ratios using elution buffer (Qiagen, United Kingdom) to generate a DNA library for sequencing on the MiSeq platform using the v2 reagent kit (Illumina Inc., United States). A commercially designed microbial community (BEI Resources, United States) was incorporated to confirm the successful recovery of the artificially designed community of bacteria.

Statistical Analysis

Data were analyzed using SPSS version 26.0 (IBM, United States). Microscopic leukocyte counts were log-transformed to accommodate for exponential distribution. The Mann–Whitney *U* test was used for numerical data, whereas Fisher's exact or $\chi 2$ test was applied to categorical data.

The 16S rRNA amplicons were processed using QIIME2 (qiime.org v2021.2.0).[47] FASTQ reads were quality-trimmed and filtered using default values, and chimera reads were identified and discarded using the R-based Divisive Amplicon Denoising Algorithm-2 (DADA2) tool. DADA2 was chosen because it relies on amplicon sequence variant classification[48] and performs well in the reconstruction of taxa relative abundances.[49] Two samples with <40,000 sequence counts were discarded. We produced a custom classifier database by generating in silico amplicons of the NCBI 16S rRNA gene database (the most suitable and reliable for urobiome studies[50]). Taxonomic assignation of amplicon reads was performed using the classify-sklearn algorithm (with default parameters) in QIIME2. Diversity analyses were performed in QIIME2, after applying a rarefaction method, retaining 42,000 reads per sample.

RESULTS

Clinical Histories and Characteristics

Urine specimens were obtained from 51 RTx patients (mean age = 50.3 y, SD = 12.9) posttransplantation (male: n = 33, female: n = 18, reflecting the ratio generally observed within this clinic) and 27 asymptomatic controls (mean age = 37.7 y, SD = 14.9) (male: n = 15, female: n = 12). We retrospectively reviewed patient clinical histories (Table S1, SDC, http://links.lww.com/TXD/A478), transplantation characteristics, pre- and posttransplantation medications (Table 1) and previous microbiological reports to ascertain culture-positive bacteriuria episodes, pre- (≤1 y before) and posttransplantation (≤1 y after). Figures S1A,B, SDC, http://links.lww.com/TXD/A478 illustrate the organisms isolated from the positive cultures of RTx patients before and after transplantation. The rate of significant bacteriuria markedly increased following

TABLE 1.

Clinical summaries of kidney transplant patients (N = 51)

Clinical summary	n (%)
Donor type	
Live donor	17 (33.3)
Deceased, unknown	2 (3.9)
Deceased, donor after brain death	20 (39.2)
Deceased, donor after circulatory death	5 (9.8)
Unknown	7 (13.7)
Renal replacement therapy	
Peritoneal dialysis	6 (11.8)
Intermittent hemodialysis	27 (52.9)
Pre-emptive transplant	11 (21.6)
Unknown	7 (13.7)
Delayed graft function	
Yes	8 (15.7)
No	36 (70.6)
Unknown	7 (13.7)
Anuric	
Yes	9 (17.6)
No	14 (27.5)
Unknown	28 (54.9)
Significant bacteriuria before transplant	
Yes [% of 'Yes' received treatment]	9 (17.6) [66.7]
No	34 (66.7)
Unknown	8 (15.7)
Recurrent episodes (% of 'Yes')	7 (77.8)
Sepsis episodes (% of 'Yes')	3 (33.3)
Significant bacteriuria on transplant day	
Yes	6 (11.8)
Significant bacteriuria after transplant	
Yes [% of 'Yes' received treatment]	29 (56.9) [55.2]
No	14 (27.5)
Unknown	8 (15.7)
Recurrent episodes [% of recurrent treated]	23 (79.3) [47.8]
Steroids	
Yes	18 (35.3)
No	27 (52.9)
Unknown	6 (11.8)
Tacrolimus	
Yes	43 (84.3)
No	2 (3.9)
Unknown	6 (11.8)

Significant bacteriuria was indicated by a positive midstream specimen of urine culture (≥10^5 colony-forming units/mL of 1 organism). The number of patients with significant bacteriuria was recorded before transplantation and for a 1-y period after transplantation.

transplantation (9 before versus 29 after, $\chi^2 = 18.860$, df = 1, $P < 0.001$). Culture reports indicated that 86.2% of patients had recurrent significant bacteriuria after transplant.

Urinary Dipstick and Microscopy

Urinary dipstick results (Table 2) revealed that 27 (52.9%) RTx patients tested positive for leukocyte esterase, which was significantly higher than the number of controls ($\chi^2 = 14.6$, df = 1, $P < 0.05$), among whom 2 (7.4%) tested positive. Five (9.8%) transplant patients were nitrite positive, whereas all controls tested negative. This difference was not significant ($\chi^2 = 2.828$, df = 1, $P = 0.093$). RTx patients had a significantly higher distribution of microscopic $\log_{10}$ leukocyte counts than controls (Mann–Whitney $U = 328.0$, $P < 0.001$) (Table 2).

Hospital Urine Culture Results

Hospital MSU culture results (Table 2) revealed that 34 (66.6%) patients had a negative culture, including 25 (49.0%) patient cultures with microbial growth of <10^5 cfu/mL. Three (5.9%) patients had mixed growth cultures, and 9 (17.7%) had a positive result (defined as 10^4–10^5 or >10^5 cfu/mL of 1 organism), among which 6 (66.7%) grew *Escherichia coli*, 2 (22.2%) grew *Enterococcus*, and 1 (11.1%) grew *Acinetobacter*.

Because mixed growth and below-threshold microbial (no significant growth or no growth) cultures are unreported by the hospital laboratory, we performed MSU cultures in parallel and identified all isolates. A significantly higher number of patients had any growth on MSU culture (n = 30) compared with controls (n = 7) ($\chi^2 = 7.66$, df = 1, $P < 0.05$) (Figure 2). A total of 8 isolates were identified from controls, whereas 37 isolates were cultured from RTx patients. Although *Streptococcus* (n = 2, 25.0%), *Escherichia* (n = 2, 25.0%), and *Staphylococcus* (n = 2, 25.0%) were most predominant among controls, *Staphylococcus* was most frequently isolated from patients (n = 19, 51.4%) and isolated at a significantly higher frequency in patients ($\chi^2 = 5.712$, df = 1, $P < 0.05$). *Enterococcus* (n = 6, 16.2%) and *Escherichia* (n = 5, 13.5%) were the next most predominantly isolated from patients.

Assessment of Concordance Across Diagnostic Tests

Across the 3 standard tests (Figure S2, SDC, http://links.lww.com/TXD/A478), concordance was observed for 14 RTx patients. Specifically, 4 (7.84%) tested positive across all tests, and ten (19.6%) tested negative (ie, no growth, no significant growth, and mixed growth cultures) across all tests. Nine (17.6%) patients were negative for dipstick nitrites and culture but positive for dipstick leukocyte esterase and had ≥10 leukocytes per microliter (WBC/μL) on microscopy. Twelve (23.5%) leukocyte esterase- and nitrite-negative patients had microscopic leukocytes, among which 3 (5.9%) had a count of ≥10 WBC/μL.

Comparison of Standard and Enhanced Culture Methods

MSU and sediment cultures were divided into 3 groups; sterile (no growth), monomicrobial (pure culture), and polymicrobial MSU (≥ 2 isolates) (Figure 3). Sediment cultures altered the microbial composition when compared with MSU cultures within patients ($\chi^2 = 40.4$, df = 2, $P < 0.001$), notably reducing sterile cultures (Post hoc: $\chi^2 = 20.3$, df = 1, $P < 0.001$) and increasing the frequency of polymicrobial cultures (Post hoc: $\chi^2 = 36.5$, df = 1, $P < 0.001$), an effect also observed in controls ($\chi^2 = 31.4$, df = 2, $P < 0.001$).

Cultured Urinary Microbial Communities

We analyzed the percentage frequencies at which genera were isolated by MSU culture and sediment culture (Figure 4A). Sediment cultures yielded a higher frequency of isolates from RTx patients (n = 109) than from controls (n = 55). With sediment culture, 10 genera were identified from controls and 12 from patients. Seven genera were shared between both groups (Figure 4B). In patients, the most abundant genera were *Staphylococcus*, *Enterococcus* and *Escherichia*, whereas *Staphylococcus*, *Enterococcus*, and *Streptococcus* were abundant in controls (Figure 4A). Despite

TABLE 2.

Clinical characteristics and routine urinalyses

Characteristic/urinalysis		Transplant patients (N = 51)	Controls (N = 27)	P
Demographics				
Female, n (%)		18 (35.3)	12 (44.4)	0.470[a]
Male, n (%)		33 (64.7)	15 (55.6)	
Mean age (SD), y		50.4 (12.9)	37.7 (14.9)	<0.001[b]
Age range, y		18–73	21–66	
Urinalyses				
Urinary dipstick, n (%)				
Leukocyte esterase positive, ≥trace		27 (52.9)	2 (7.4)	<0.001[a]
Leukocyte esterase negative		24 (47.1)	25 (92.6)	
Nitrite positive		5 (9.8)	0 (0.0)	0.157[a]
Nitrite negative		46 (90.2)	27 (100.0)	
Urinary WBC microscopy				
Mean $\log_{10}$ WBC count, SD (95% CI)		0.84, 0.93 (0.58-1.10)	0.13, 0.30 (0.01-0.25)	<0.001[b]
Median $\log_{10}$ WBC count (95% CI)		0.60 (0.30-1.00)	0.00 (0.00-0.00)	
WBC frequencies, n (%)				
Zero WBC/μL		17 (33.3)	21 (77.8)	<0.001[a]
1–9 WBC/μL		15 (29.4)	5 (18.5)	
≥10 WBC/μL		19 (37.3)	1 (3.7)	
Routine MSU culture, n (%)				
Missing		5 (9.8)		
Negative	No growth	9 (17.6)		
	No significant growth, $<10^5$ cfu/mL	25 (49.0)		
	Mixed growth	3 (5.9)		
Positive	10^4–10^5 cfu/mL of 1 organism	1 (2.0)		
	10^5 cfu/mL of 1 organism	8 (15.7)		
Microbe identified on positive culture	*Escherichia coli*	6 (66.7)		
	Acinetobacterjunii	1 (11.1)		
	Enterococcus faecalis	1 (11.1)		
	Enterococcus faecium	1 (11.1)		

[a]χ^2 test.
[b]Mann–Whitney *U* test.
CI, confidence interval; cfu/mL, colony-forming units per milliliter; MSU, midstream specimen of urine; WBC, white blood cells.

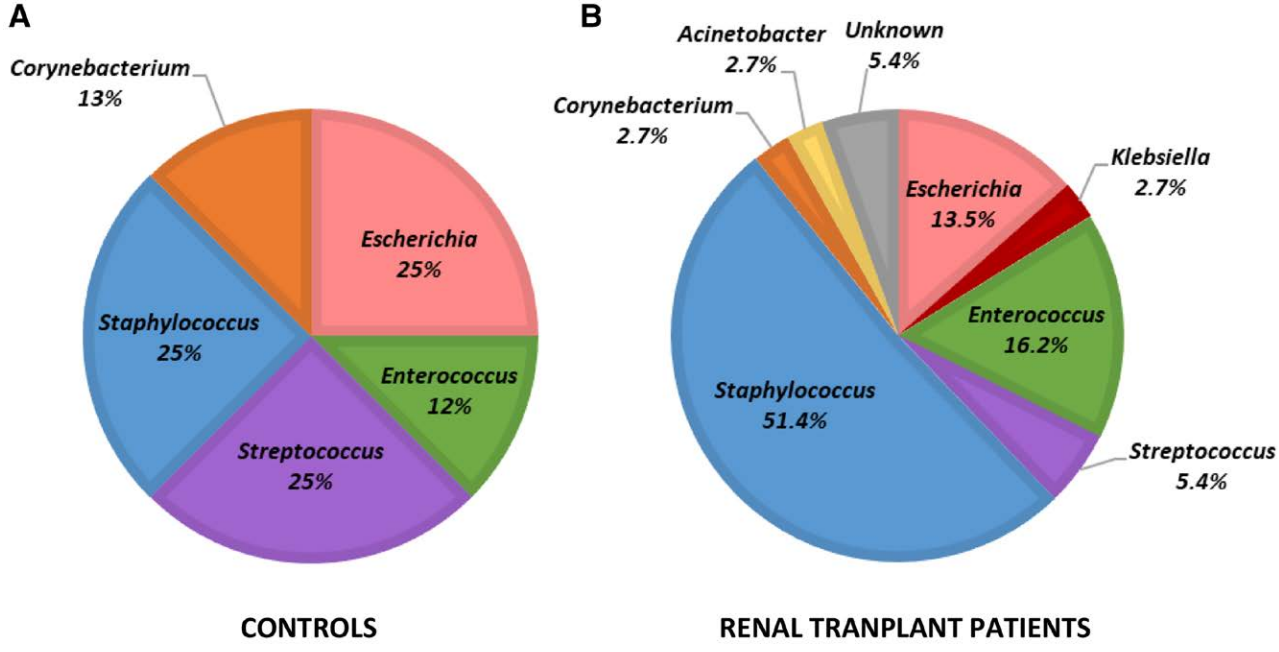

FIGURE 2. Percentage frequencies of isolates (genus level) identified from control (A; n = 7) and renal transplant patient (B; n = 30) midstream urine cultures performed at University College London research laboratories.

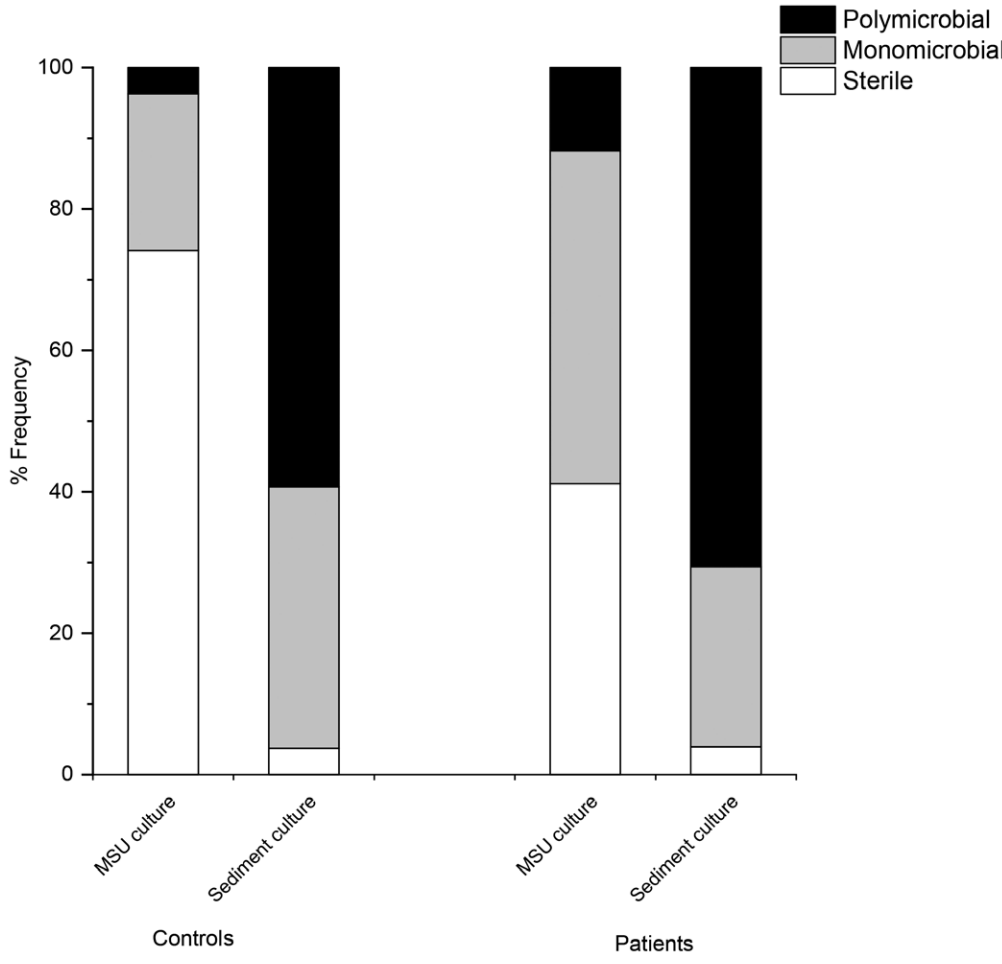

FIGURE 3. Percentage frequencies of sterile (no microbial growth), pure (monomicrobial growth) and mixed (polymicrobial) growth cultures identified with the MSU culture and enhanced sediment culture techniques. MSU, midstream specimen of urine.

differences in the abundances of genera on sediment culture between both groups, these were not statistically significant. At species-level (Figure 5), *Staphylococcus haemolyticus* (*S. haemolyticus*), *Staphylococcus epidermidis* (*S. epidermidis*), *E. coli*, and *Enterococcus faecalis (E. faecalis)* were most frequently cultured in patients, whereas *S. haemolyticus*, *E. faecalis*, and *S. epidermidis* dominated in controls. We found a significant difference in the frequency of *Streptococcus agalactiae* in controls, in contrast to their absence in patients ($\chi^2 = 8.120$, df = 1, $P < 0.05$).

Poly-pie chart matrices of sediment cultures were constructed to provide a detailed view of the urinary microbial composition within each subject (Figure 6A,B). No significant difference was identified between the number of patient (n = 36, 70.6%) and control (n = 16, 59.3%) polymicrobial sediment cultures ($\chi^2 = 1.020$, df = 1, $P = 0.313$). We assessed the mono- and polymicrobial nature of each genus and species on sediment culture. Regardless of study group, *Enterococcus* ($\chi^2 = 7.065$, df = 1, $P < 0.05$) and *E. coli* ($\chi^2 = 6.132$, df = 1, $P < 0.05$) were more frequently isolated from polymicrobial cultures than from monomicrobial cultures. Within patients, *Enterococcus* ($\chi^2 = 12.203$, df = 1, $P < 0.001$) and *Staphylococcus* ($\chi^2 = 5.105$, df = 1, $P < 0.05$), notably *E. faecalis* ($\chi^2 = 8.579$, df = 1, $P < 0.05$) and *S. haemolyticus* ($\chi^2 = 10.156$, df = 1, $P < 0.05$), were more frequently identified on polymicrobial than monomicrobial cultures. Within controls, *Staphylococcus* was more commonly polymicrobial ($\chi^2 = 5.105$, df = 1, $P < 0.05$).

Next, we compared the frequencies at which each genus and species presented itself as a monomicrobial or polymicrobial isolate between study groups. A significantly higher number of polymicrobial *Enterococcus* isolates ($\chi^2 = 9.231$, df = 1, $P < 0.05$), particularly *E. faecalis* ($\chi^2 = 6.061$, df = 1, $P < 0.05$) were seen with patients than with controls.

The most commonly observed combinations of organisms on sediment cultures were *E. coli* with *S. epidermidis* (n = 7), *E. faecalis* with *S. haemolyticus* (n = 6) and *E. coli* with *E. faecalis* (n = 5) in patients and *E. faecalis* with *S. epidermidis* (n = 3) in controls.

Sequenced Urinary Bacterial Communities

We analyzed the bacterial communities of 51 patients and 20 controls by sequencing the V3-V4 regions of the 16S rRNA gene from urine specimens. Bacterial DNA was recovered from 45 (88.2%) RTx patients and 12 (60.0%) controls. The percentage of identified sequencing reads that passed filter ([PF], Q30 >70%) for the sequenced DNA library was 82.26% (26,913,200 reads).

The 15 most abundant genera across both study groups represented 87.8% of all control sequences and 87.9% of all patient sequences (Figure 7). The remaining sequences were grouped as "Other." *Gardnerella* (18.9%), *Escherichia*

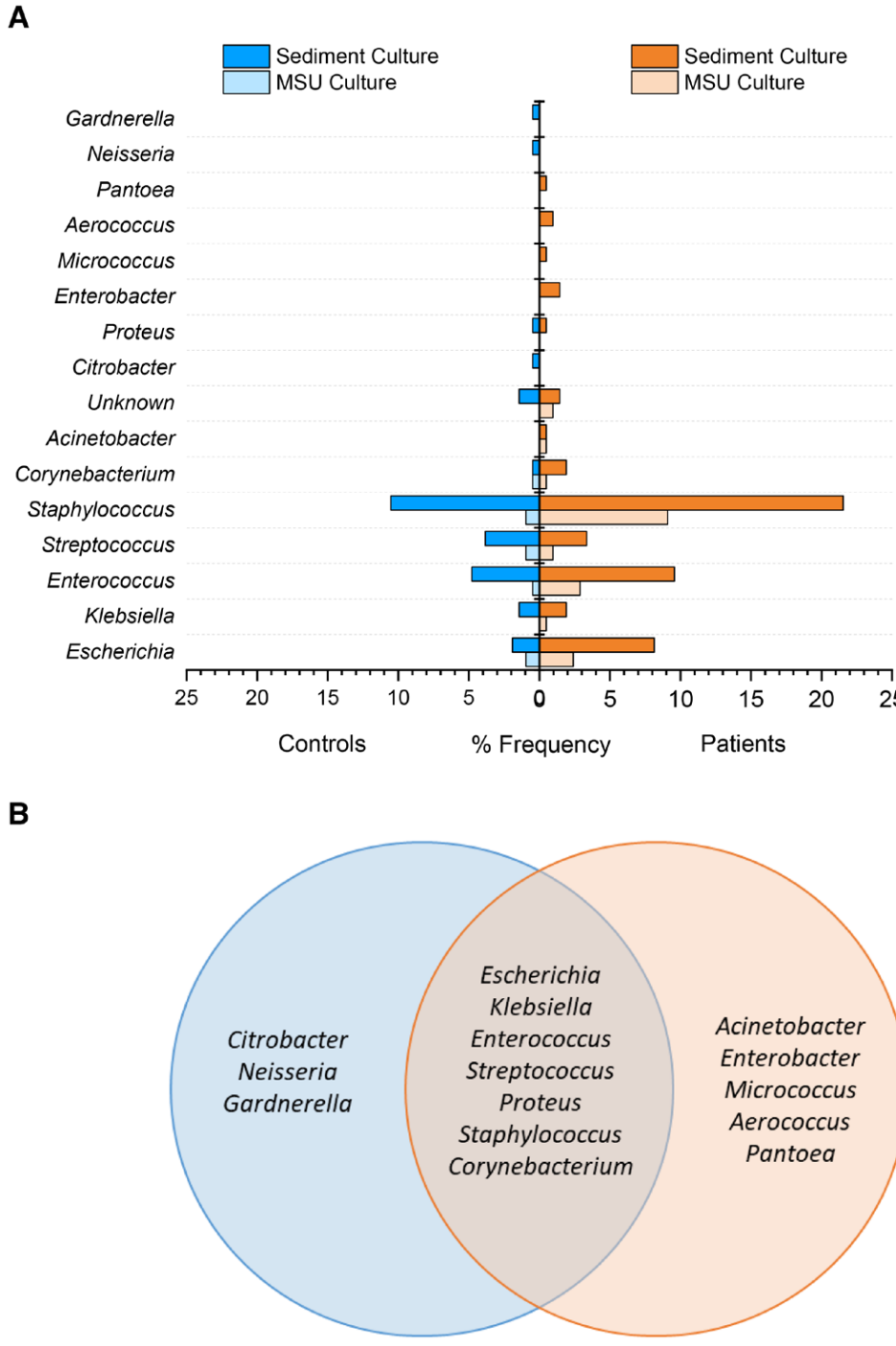

FIGURE 4. Genera isolated from renal transplant patients and controls. A, Percentage frequencies of organisms identified from the urine specimens of renal transplant patients (N = 51) and healthy controls (N = 27) using the routine MSU culture and centrifuged sediment culture techniques. Data are presented as percentages of the total number of isolates identified from all cultures. B, Venn diagram portraying the genera isolated exclusively from transplant patients, controls and those shared by both groups. MSU, midstream specimen of urine.

(17.2%), and *Lactobacillus* (17.0%) were most abundant in patients; and in controls, *Lactobacillus* (52.9%), followed by *Streptococcus* (11.9%) and *Gardnerella* (6.65%). A significantly higher number of control samples contained *Flavobacterium* ($\chi^2 = 5.31$, df = 1, $P < 0.05$). Additionally, we observed the 10 most abundant taxa in each patient and control sample (Figure 8), which were assigned to 1 of 12 urotypes, defined by the dominant genus (Figure S3, SDC, http://links.lww.com/TXD/A478).[29] *Gardnerella* and *Corynebacterium* urotypes were associated almost exclusively with male patients, whereas patient samples with >80% of sequences assigned to *Gardnerella* showed 2 or more positive UTI tests.

Comparison of Cultured and Sequenced Communities

We compared the isolation capabilities of both sediment culture and 16S rRNA sequencing for patient and control urines

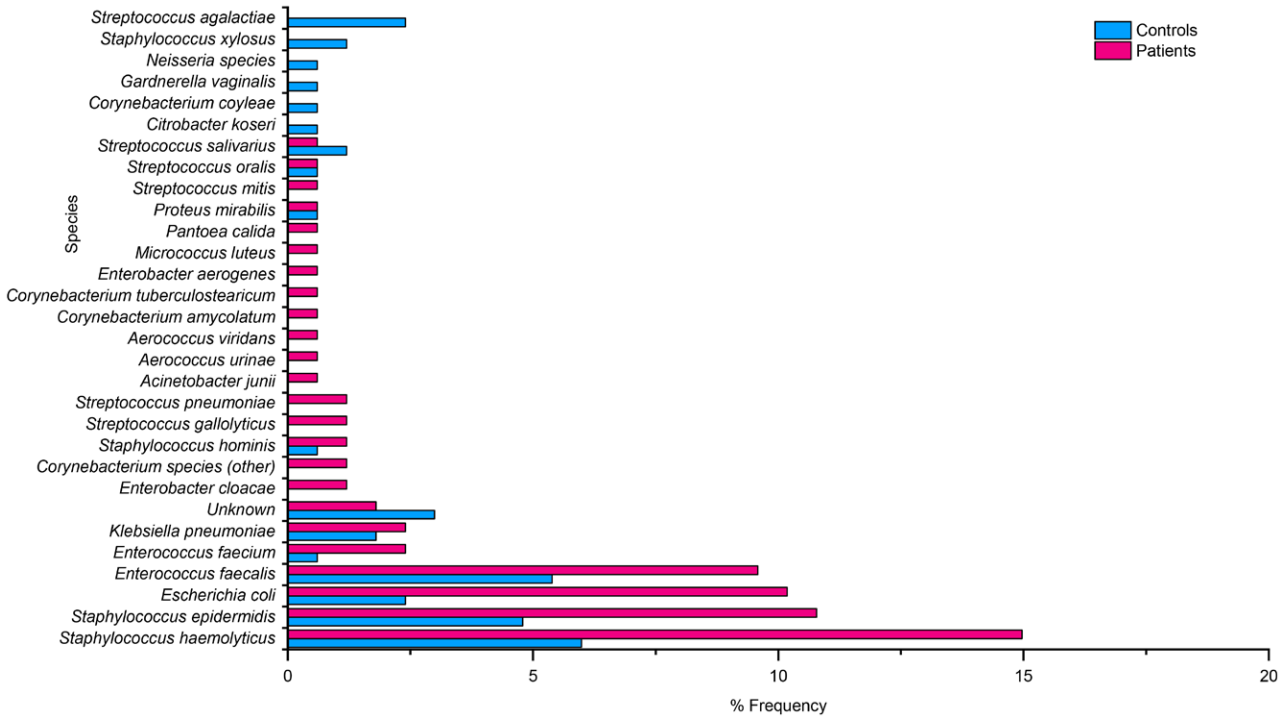

FIGURE 5. Percentage frequency of microbial species isolated from renal transplant patient and control sediment cultures. Data are presented as percentages of total number of isolates identified from sediment cultures.

(Figure 9). In a total of 57 samples, 47 (82.5%) contained genera that were identified by both sediment culture and sequencing. Thirty samples contained genera that were identified by culture but not by sequencing. *Corynebacterium* was the most frequently isolated genus by sequencing, whereas *Staphylococcus* was most frequently identified by both techniques. One patient sample grew *Pantoea,* and 1 patient grew *Proteus* on sediment culture, which were not detected by sequencing.

Evidence of Bacterial Association and Invasion of Urothelial Cells

We inspected patient and control urine samples by epifluorescence microscopy after staining for DNA and uroplakin IIIa protein to identify any bacterial association with urothelial cells (Figure 10 shows a representative example from a patient). Among patients, 16 (31.4%) showed evidence of bacteria-associated shed cells, whereas 6 (11.8%) controls had bacteria-associated epithelial cells. This difference was not significant ($\chi^2 = 0.730$, df = 1, $P = 0.393$).

DISCUSSION

The Diagnostic Conundrum of RTx Patients

UTI detection currently relies on traditional diagnostic tests with recognized limitations.[25-28] It is only relatively recently that genomic approaches revealed that the healthy urinary tract is inhabited by a complex microbiota; although this community may be disrupted in UTI, it also complicates diagnosis.[28,29,33,35] Although the performance (and improvement) of diagnostic tests and characterization of the urobiome in different patient groups are active research areas,[24,25,28,29,51] these appear to be severely understudied in RTx patients, who have a particularly urgent need for early detection and appropriate treatment of UTI to prevent serious consequences.[9,11,17,19]

In this study, RTx patient histories revealed an increase in culture-positive bacteriuria after transplantation, which is a primary indicator of potential urinary tract microbial dysbiosis. Posttransplantation, these patients also experienced a high rate of recurrence (86.2%). These characteristics reinforce the notion that RTx patients, as reported in the literature, are prone to UTI.[3-6] To learn more, we performed a cross-sectional study of posttransplant RTx patients and heathy controls to (i) compare the diagnostic culture with alternative techniques and (ii) characterize RTx patient urinary microbial communities.

Study Scope and Limitations

Limitations of this study included the lack of symptomatic assessment, which is important in distinguishing between ASB and UTI in uncomplicated patients. However, this step is not so straightforward in immunosuppressed RTx patients, further complicating the decision to treat ASB, for which there is currently no formal consensus. In addition to small sample sizes, the mean ages of patient and control groups were significantly different. Although 9 female RTx patients and 3 female controls were aged ≥50 y, the menopausal status of female subjects, a known influencer of the urobiome, was unknown. Although this study used random patient recruitment to reflect the clinic's general demographics, future studies should consider urobiome determinants, namely sex,[33,52] age,[33] and menopausal status.[53] In this study, cultures and conditions favorable toward anaerobes were not performed. Previous work has shown that the use of an extensive range of culture media and conditions increases the species diversity observed.[24,28,29,54] Here, we sought to determine if the sediment culture technique, designed to enhance microbial isolation (including organisms associated with urothelial cells) improved the MSU culture's performance while using

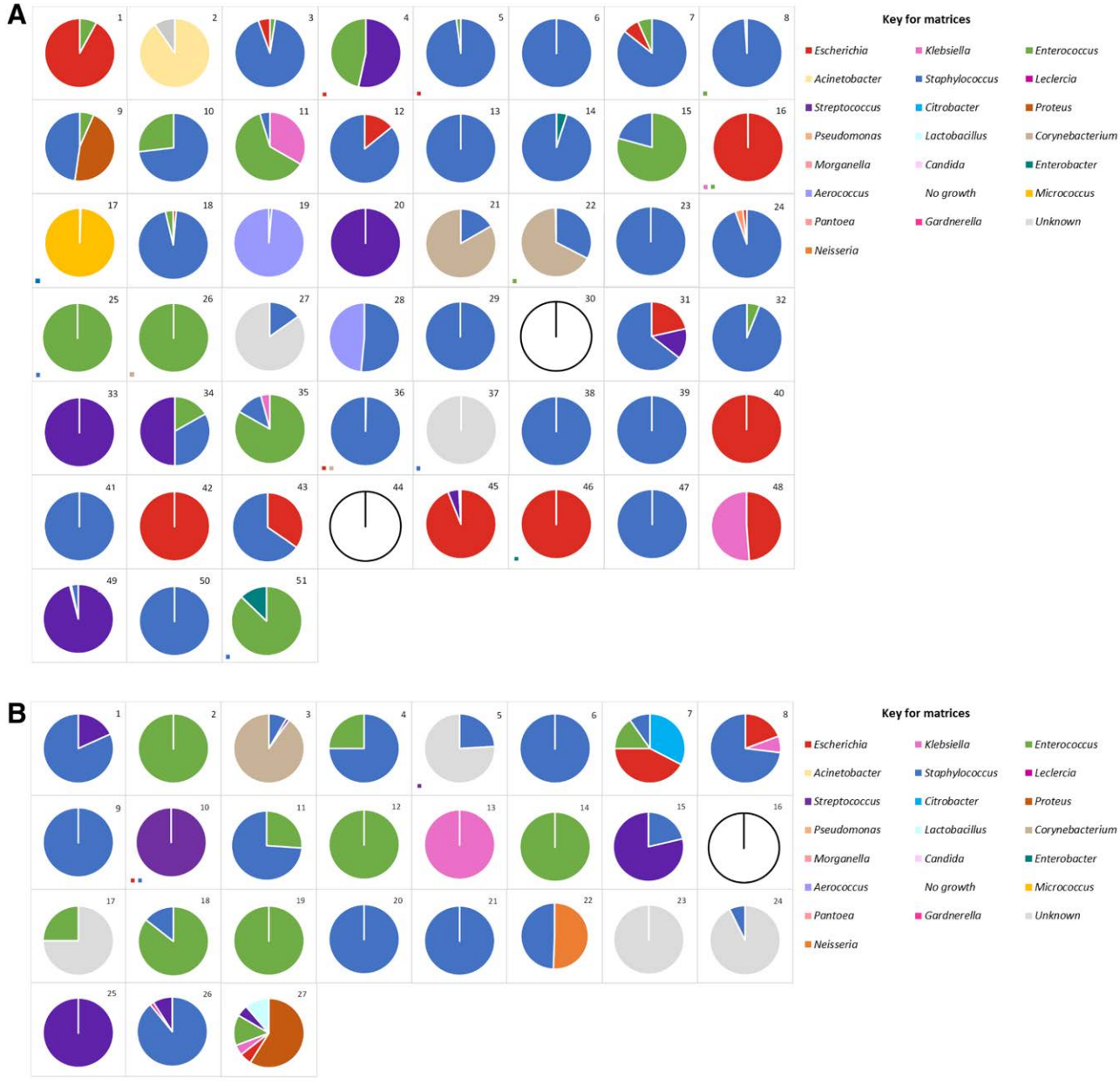

FIGURE 6. Poly-pie matrix portraying microbial compositions of sediment cultures. A, Microbial compositions for renal transplant patients (N = 51). B, Microbial compositions for asymptomatic controls (N = 27). Microbial frequencies of <1% are indicated by small colored icon under each pie.

identical culture and incubation conditions. We employed sequencing not only to further highlight the bacteria being missed by culture but also to provide a more complete view of the urinary tract bacterial community. Despite these limitations, our analysis provides the first assessment of standard diagnostics with characterization of urinary microbiota in posttransplant RTx patients, facilitating hypotheses for larger follow-up studies.

Comparison of Diagnostic Techniques

Throughout this study, we identified challenges to UTI detection. The significantly elevated inflammatory signal observed with dipstick leukocyte esterase and microscopic leukocytes might not be considered unusual in RTx patients, but given the sheer prevalence of posttransplant UTI, undiagnosed infection (or beginnings of one) is another possibility—a crucial distinction when clinicians are relying on a combination of diagnostic tests and clinical presentation. In this study, the inability to distinguish between infection and inflammation was unaided by the discordance observed across dipstick, microscopy, and culture results, reaffirming their unreliability.[25-27] This raises some unanswered questions: (i) Is microbial dysbiosis expected posttransplantation? (ii) Is dysbiosis temporary and how long should it last? (iii) How would the transplant dysbiotic state differ from that observed with UTI development? and (iv) At what stage should there be intervention?

Our sediment culture and sequencing also highlighted known deficiencies in the diagnostic MSU culture and its accompanying criteria (presence of $\geq 10^5$ cfu/mL of one uropathogen),[30-32] indicating that this method underestimated both microbial growth and its predominantly polymicrobial

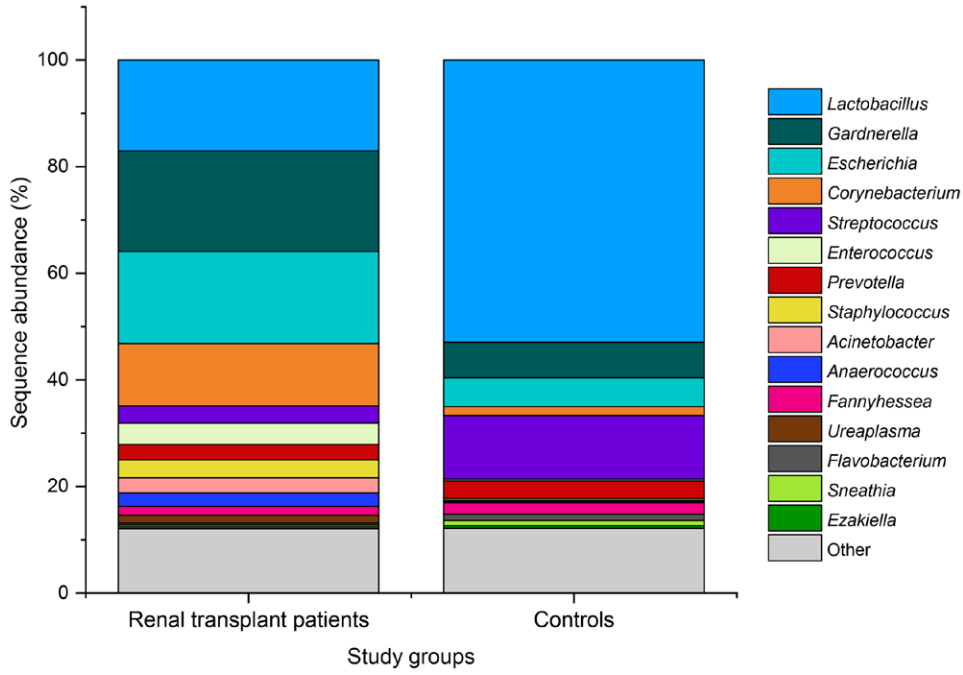

FIGURE 7. Percentage sequence abundances of the 15 most abundant taxa detected in renal transplant patients and control study groups.

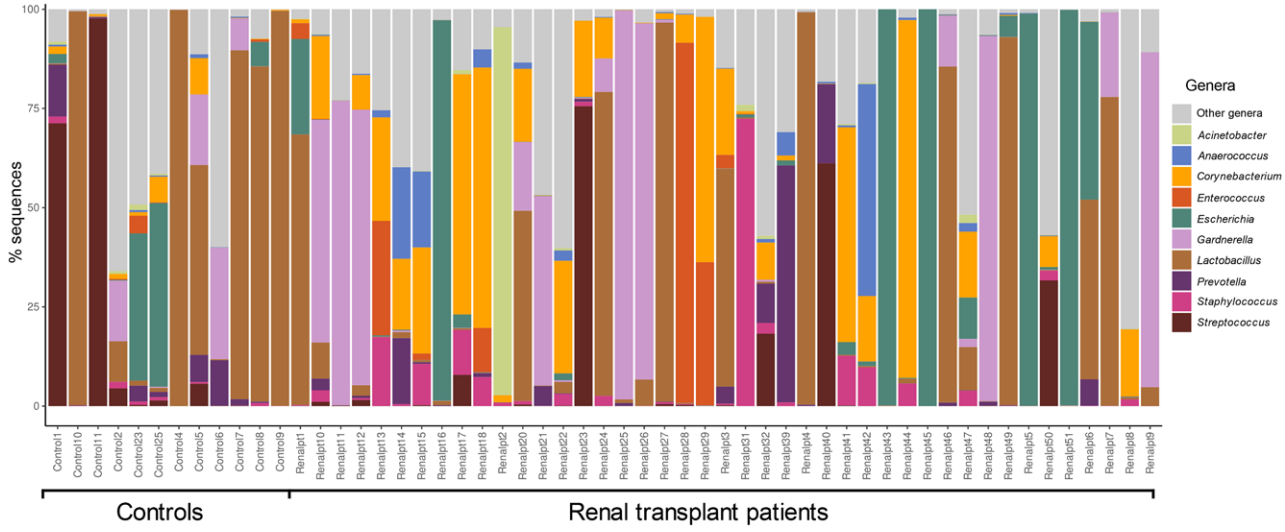

FIGURE 8. Individual-level stacked plot showing the relative abundances of the 10 most abundant taxa in each renal transplant patient and control.

nature and thus, may be misleading. Given our observations that some bacteria are host cell associated (Figure 10), sediment culture would be expected to enrich for such species, compared with MSU culture. Others have also successfully employed expanded quantitative urine culture protocols for microbial enrichment.[24,28,29,51,54-56] Our comparison of taxa identified by culture and sequencing confirms that each approach carries limitations and biases (Figure 9). Although bacterial growth on culture is determined by the choices researchers make concerning media and laboratory conditions, it permits strain-level identification, characterization, and susceptibility testing. On the other hand, 16S rRNA sequencing reveals more species richness compared with expansive culture but can fail to identify organisms because of vagaries in DNA extraction, PCR parameters, amplicon clean-up, and analysis. However, the development of effective diagnostics also relies on improved understanding of the community-based pathophysiological processes leading to UTI development. In the meantime, our results support the conclusion also shown by others that optimized culture and sequencing combined improves resolution of community richness.[28,54,57,58]

Insights Into the Microbial Communities of RTx Patients

Our study also allowed us to inspect differences in the urobiome between RTx patients and controls. The existence of a healthy urinary microbiota and its polymicrobial nature[28,29,33,35,36] complicates the identification of which species are involved in UTI. This is evident from the overlap in

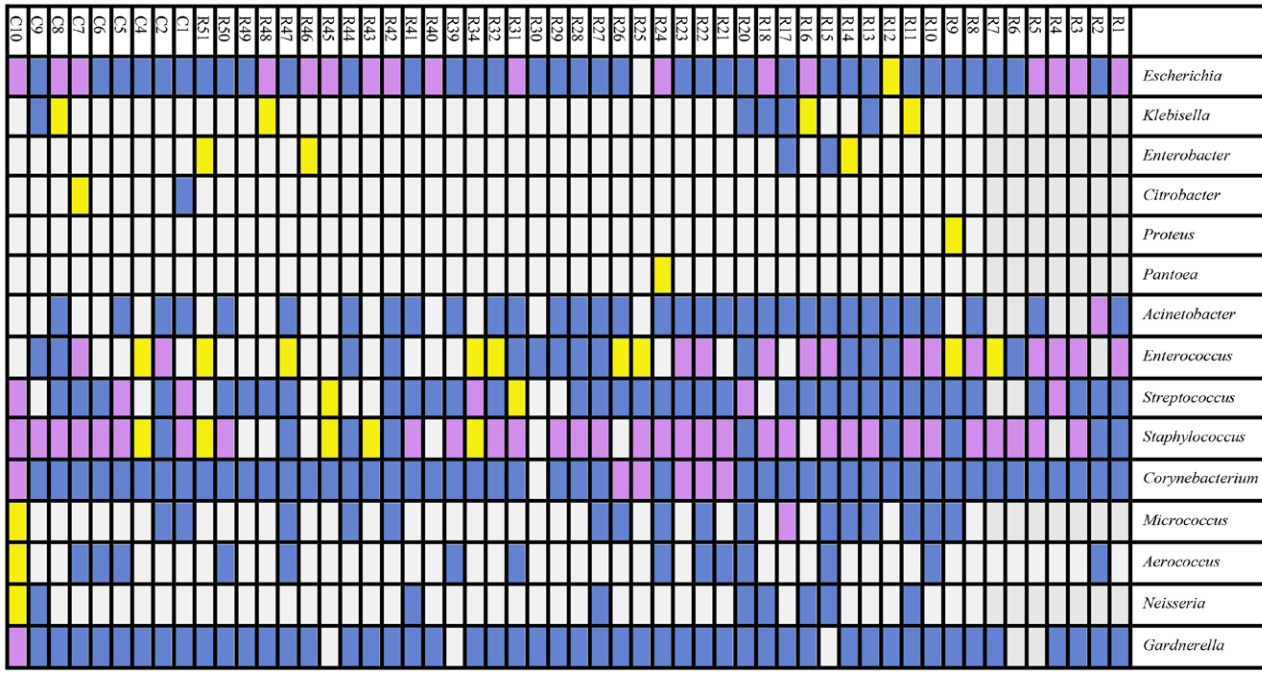

FIGURE 9. Color-coded matrix of taxa that were identified by (i) culture only (yellow), (ii) sequencing only (blue), (iii) both approaches (purple), and (iv) neither technique (light gray) from renal transplant patient (n = 45) and control (n = 12) urine.

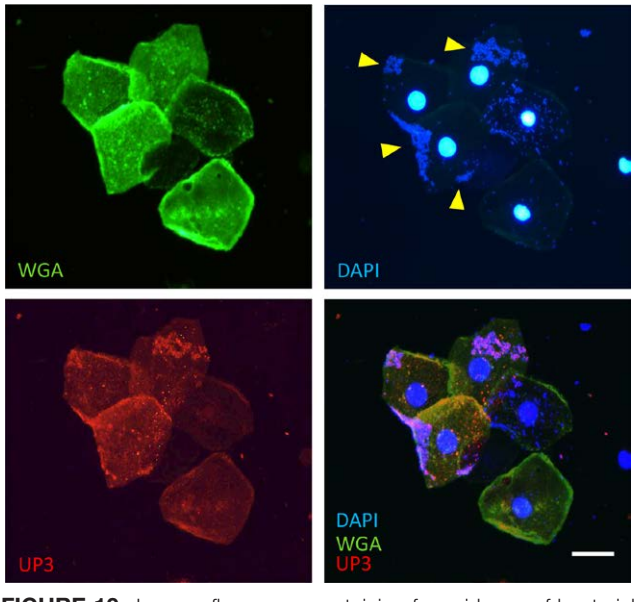

FIGURE 10. Immunofluorescence staining for evidence of bacterial association with uroplakin-positive urothelial cells in a renal transplant patient urine sample. A, WGA, a host cell membrane stain; (B) DAPI, which stains the DNA of the host cell nucleus (brightest blue ovals) and of the bacteria (much smaller blue bodies covering all cells; yellow arrows indicate some particularly large clusters of bacteria); (C) UP3 antibody, a marker specific for urothelial cells; and (D) a merge of the 3 previous channels. Scale bar is 30 µm. DAPI, 4',6-diamidino-2-phenylindole; UP3, uroplakin III; WGA, wheat germ agglutinin.

predominant organisms identified on patient and control sediment cultures (Figure 4), which consisted of *Staphylococcus*, *Enterococcus*, and *E. coli*, which were also the most commonly co-cultured. Indeed, *E. coli* and *E. faecalis* are recognized for their dual appearance in urinary specimens.[59,60]

Characterization of RTx patient urine using 16S rRNA sequencing revealed that *Gardnerella*, *Escherichia*, and *Lactobacillus* were most abundant, with some abundant taxa shared by controls (Figure 7). However, *Flavobacterium* was significantly more frequent in controls. Such urobiome differences may be attributed to the combined and poorly understood effects of immunosuppressants, antibiotics,[38,61] and steroids in patients. They may also or alternatively reflect meaningful UTI dysbiosis. Profiling urobiome genomic and functional capabilities may reveal key characteristics specific to non-UTI and UTI states in RTx patients.[62,63]

We identified an as-yet-unreported potential association of *Gardnerella* and *Corynebacterium* urotypes assigned to RTx patients, nearly all male. *Corynebacterium* has previously been associated with allograft dysfunction in RTx patients.[64] Patient samples containing >80% of sequences assigned to *Gardnerella* exhibited 2 or more positive diagnostic tests, raising the possibility that *Gardnerella* may be involved in UTI development and inflammation in RTx patients. *Gardnerella* (notably *Gardnerella vaginalis*) is traditionally associated with bacterial vaginosis but is also reported in vaginal and urine samples of healthy women.[65-68] *G. vaginalis* has been shown to retrigger recurrent UPEC-induced UTI in mice.[69] Also of interest may be *Gardnerella*'s covert prevalence in men with inflammation observed on prostate biopsies[70] and its status as an uncommon uropathogen for immunocompromised and RTx patients.[71] In our cohort, it is unclear whether *Gardnerella* is associated with infection, inflammation, or both, but further investigation is warranted.

CONCLUSIONS

This study highlights the serious inadequacies of traditional UTI diagnostics in the post-RTx cohort, whereas providing insights into their clinical and microbiological complexities. Future, in-depth urobiome studies may allow the development of an "early warning system" for the detection of future problematic UTIs.

Although our techniques improved microbial isolation and provided insights into their polymicrobial nature, future longitudinal studies profiling microbial dynamics during pre- and posttransplantation stages may provide clarity on the urobiome's role in RTx patients. Additionally, the inclusion and profiling of kidney donors may provide answers on how the urobiome is defined in corresponding recipients. It would also be valuable to consider the interplay of viruses and fungi, investigate associated niches (ie, gut[72-74]), as well as interactions with the immune system.[75] More targeted therapeutics, facilitated by detailed understanding of the dynamic urinary tract microbial ecology, may reduce the need for prophylactic antibiotics. This, in turn, would aid in antibiotic stewardship and reduce or prevent collateral harm to the human microbiome, which may have long-term implications for RTx patients.

ACKNOWLEDGMENTS

We are grateful to Neil Jones and Steven De'Ath (Microbiology Department, Whittington Hospital, United Kingdom) for their technical support with MALDI-TOF MS-based microbial identification, and Tony Brooks (Genomics Centre, University College London, United Kingdom) for his assistance with the use of the MiSeq platform. We also thank Stephen Mepham and Alan Salama for their helpful discussions and review of this work. We would also like to thank the referees of an earlier version of this article for their constructive and helpful comments.

The following reagent was obtained through BEI Resources, NIAID, NIH as part of the Human Microbiome Project: Genomic DNA from Microbial Mock Community B (Even, Low Concentration), v5.1L, for 16S rRNA Gene Sequencing, HM-782D.

REFERENCES

1. Harding GKM, Ronald AR. The management of urinary infections; what have we learned in the past decade? *Int J Antimicrob Agents*. 1994;4:83–88.
2. Flores-Mireles AL, Walker JN, Caparon M, et al. Urinary tract infections: epidemiology, mechanisms of infection and treatment options. *Nat Rev Microbiol*. 2015;13:269–284.
3. Bodro M, Linares L, Chiang D, et al. Managing recurrent urinary tract infections in kidney transplant patients. *Expert Rev Anti Infect Ther*. 2018;16:723–732.
4. Alangaden GJ, Thyagarajan R, Gruber SA, et al. Infectious complications after kidney transplantation: current epidemiology and associated risk factors. *Clin Transplant*. 2006;20:401–409.
5. Hollyer I, Ison MG. The challenge of urinary tract infections in renal transplant recipients. *Transpl Infect Dis*. 2018;20:e12828.
6. Valera B, Gentil MA, Cabello V, et al. Epidemiology of urinary infections in renal transplant recipients. *Transplant Proc*. 2006;38:2414–2415.
7. Al Midani A, Elands S, Collier S, et al. Impact of urinary tract infections in kidney transplant recipients: a 4-year single-center experience. *Transplant Proc*. 2018;50:3351–3355.
8. Naik AS, Dharnidharka VR, Schnitzler MA, et al. Clinical and economic consequences of first-year urinary tract infections, sepsis, and pneumonia in contemporary kidney transplantation practice. *Transpl Int*. 2016;29:241–252.
9. Graversen ME, Dalgaard LS, Jensen-Fangel S, et al. Risk and outcome of pyelonephritis among renal transplant recipients. *BMC Infect Dis*. 2016;16:264.
10. Ariza-Heredia EJ, Beam EN, Lesnick TG, et al. Impact of urinary tract infection on allograft function after kidney transplantation. *Clin Transplant*. 2014;28:683–690.
11. Säemann M, Hörl WH. Urinary tract infection in renal transplant recipients. *Eur J Clin Invest*. 2008;38:58–65.
12. Bodro M, Sanclemente G, Lipperheide I, et al. Impact of urinary tract infections on short-term kidney graft outcome. *Clin Microbiol Infect*. 2015;21:1104.e1–8.
13. Britt NS, Hagopian JC, Brennan DC, et al. Effects of recurrent urinary tract infections on graft and patient outcomes after kidney transplantation. *Nephrol Dial Transplant*. 2017;32:1758–1766.
14. Shendi AM, Wallis G, Painter H, et al. Epidemiology and impact of bloodstream infections among kidney transplant recipients: a retrospective single-center experience. *Transpl Infect Dis*. 2018;20:e12815.
15. Silva M, Jr, Marra AR, Pereira CA, et al. Bloodstream infection after kidney transplantation: epidemiology, microbiology, associated risk factors, and outcome. *Transplantation*. 2010;90:581–587.
16. Lee JR, Bang H, Dadhania D, et al. Independent risk factors for urinary tract infection and for subsequent bacteremia or acute cellular rejection: a single-center report of 1166 kidney allograft recipients. *Transplantation*. 2013;96:732–738.
17. Trzeciak S, Sharer R, Piper D, et al. Infections and severe sepsis in solid-organ transplant patients admitted from a university-based ED. *Am J Emerg Med*. 2004;22:530–533.
18. Pellé G, Vimont S, Levy PP, et al. Acute pyelonephritis represents a risk factor impairing long-term kidney graft function. *Am J Transplant*. 2007;7:899–907.
19. Chuang P, Parikh CR, Langone A. Urinary tract infections after renal transplantation: a retrospective review at two US transplant centers. *Clin Transplant*. 2005;19:230–235.
20. Abbott KC, Swanson SJ, Richter ER, et al. Late urinary tract infection after renal transplantation in the United States. *Am J Kidney Dis*. 2004;44:353–362.
21. Silva C, Afonso N, Macário F, et al. Recurrent urinary tract infections in kidney transplant recipients. *Transplant Proc*. 2013;45:1092–1095.
22. Velioglu A, Guneri G, Arikan H, et al. Incidence and risk factors for urinary tract infections in the first year after renal transplantation. *PLoS One*. 2021;16:e0251036.
23. Camargo LF, Esteves AB, Ulisses LR, et al. Urinary tract infection in renal transplant recipients: incidence, risk factors, and impact on graft function. *Transplant Proc*. 2014;46:1757–1759.
24. Price TK, Dune T, Hilt EE, et al. The clinical urine culture: enhanced techniques improve detection of clinically relevant microorganisms. *J Clin Microbiol*. 2016;54:1216–1222.
25. Sathiananthamoorthy S, Malone-Lee J, Gill K, et al. Reassessment of routine midstream culture in diagnosis of urinary tract infection. *J Clin Microbiol*. 2019;57:e01452–e01418.
26. Khasriya R, Khan S, Lunawat R, et al. The inadequacy of urinary dipstick and microscopy as surrogate markers of urinary tract infection in urological outpatients with lower urinary tract symptoms without acute frequency and dysuria. *J Urol*. 2010;183:1843–1847.
27. Kupelian AS, Horsley H, Khasriya R, et al. Discrediting microscopic pyuria and leucocyte esterase as diagnostic surrogates for infection in patients with lower urinary tract symptoms: results from a clinical and laboratory evaluation. *BJU Int*. 2013;112:231–238.
28. Hilt EE, McKinley K, Pearce MM, et al. Urine is not sterile: use of enhanced urine culture techniques to detect resident bacterial flora in the adult female bladder. *J Clin Microbiol*. 2014;52:871–876.
29. Pearce MM, Hilt EE, Rosenfeld AB, et al. The female urinary microbiome: a comparison of women with and without urgency urinary incontinence. *mBio*. 2014;5:e01283–e01214.
30. Kass EH. Asymptomatic infections of the urinary tract. *Trans Assoc Am Physicians*. 1956;69:56–64.
31. Kass EH. Bacteriuria and the diagnosis of infections of the urinary tract; with observations on the use of methionine as a urinary antiseptic. *AMA Arch Intern Med*. 1957;100:709–714.
32. Public Health England. UK standards for microbiology investigations. *Bacteriology*. 2019;8:1–51.
33. Lewis DA, Brown R, Williams J, et al. The human urinary microbiome; bacterial DNA in voided urine of asymptomatic adults. *Front Cell Infect Microbiol*. 2013;3:41.
34. Wolfe AJ, Toh E, Shibata N, et al. Evidence of uncultivated bacteria in the adult female bladder. *J Clin Microbiol*. 2012;50:1376–1383.
35. Siddiqui H, Nederbragt AJ, Lagesen K, et al. Assessing diversity of the female urine microbiota by high throughput sequencing of 16S rDNA amplicons. *BMC Microbiol*. 2011;11:244.
36. Fouts DE, Pieper R, Szpakowski S, et al. Integrated next-generation sequencing of 16S rDNA and metaproteomics differentiate the healthy urine microbiome from asymptomatic bacteriuria in neuropathic bladder associated with spinal cord injury. *J Transl Med*. 2012;10:174.

37. Fricke WF, Maddox C, Song Y, et al. Human microbiota characterization in the course of renal transplantation. *Am J Transplant*. 2014;14:416–427.
38. Rani A, Ranjan R, McGee HS, et al. Urinary microbiome of kidney transplant patients reveals dysbiosis with potential for antibiotic resistance. *Transl Res*. 2017;181:59–70.
39. Hugenholtz F, van der Veer C, Terpstra ML, et al. Urine and vaginal microbiota compositions of postmenopausal and premenopausal women differ regardless of recurrent urinary tract infection and renal transplant status. *Sci Rep*. 2022;12:2698.
40. Coussement J, Kamar N, Matignon M, et al; Bacteriuria in Renal Transplantation (BiRT) study group. Bacteriuria in Renal Transplantation (BiRT) study group. Antibiotics versus no therapy in kidney transplant recipients with asymptomatic bacteriuria (BiRT): a pragmatic, multicentre, randomized, controlled trial. *Clin Microbiol Infect*. 2021;27:398–405.
41. Sabé N, Oriol I, Melilli E, et al. Antibiotic treatment versus no treatment for asymptomatic bacteriuria in kidney transplant recipients: a multicenter randomized trial. *Open Forum Infect Dis*. 2019;6:ofz243.
42. Origüen J, López-Medrano F, Fernández-Ruiz M, et al. Should asymptomatic bacteriuria be systematically treated in kidney transplant recipients? Results from a randomized controlled trial. *Am J Transplant*. 2016;16:2943–2953.
43. Fiorentino M, Pesce F, Schena A, et al. Updates on urinary tract infections in kidney transplantation. *J Nephrol*. 2019;32:751–761.
44. Fiorante S, López-Medrano F, Lizasoain M, et al. Systematic screening and treatment of asymptomatic bacteriuria in renal transplant recipients. *Kidney Int*. 2010;78:774–781.
45. Horsley H, Malone-Lee J, Holland D, et al. *Enterococcus faecalis* subverts and invades the host urothelium in patients with chronic urinary tract infection. *PLoS One*. 2013;8:e83637.
46. Yuan S, Cohen DB, Ravel J, et al. Evaluation of methods for the extraction and purification of DNA from the human microbiome. *PLoS One*. 2012;7:e33865.
47. Bolyen E, Rideout JR, Dillon MR, et al. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nat Biotechnol*. 2019;37:852–857.
48. Brubaker L, Gourdine JF, Siddiqui NY, et al. Forming consensus to advance urobiome research. *mSystems*. 2021;6:e0137120.
49. Nearing JT, Douglas GM, Comeau AM, et al. Denoising the denoisers: an independent evaluation of microbiome sequence error-correction approaches. *Peer J*. 2018;6:e5364.
50. Hoffman C, Siddiqui NY, Fields I, et al. Species-level resolution of female bladder microbiota from 16S rRNA amplicon sequencing. *mSystems*. 2021;6:e0051821.
51. Coorevits L, Heytens S, Boelens J, et al. The resident microflora of voided midstream urine of healthy controls: standard versus expanded urine culture protocols. *Eur J Clin Microbiol Infect Dis*. 2017;36:635–639.
52. Gottschick C, Deng ZL, Vital M, et al. The urinary microbiota of men and women and its changes in women during bacterial vaginosis and antibiotic treatment. *Microbiome*. 2017;5:99.
53. Ammitzbøll N, Bau BPJ, Bundgaard-Nielsen C, et al. Pre- and postmenopausal women have different core urinary microbiota. *Sci Rep*. 2021;11:2212.
54. Vaughan MH, Mao J, Karstens LA, et al. The urinary microbiome in postmenopausal women with recurrent urinary tract infections. *J Urol*. 2021;206:1222–1231.
55. Barnes HC, Wolff B, Abdul-Rahim O, et al. A randomized clinical trial of standard versus expanded cultures to diagnose urinary tract infections in women. *J Urol*. 2021;206:1212–1221.
56. Burnett LA, Hochstedler BR, Weldon K, et al. Recurrent urinary tract infection: association of clinical profiles with urobiome composition in women. *Neurourol Urodyn*. 2021;40:1479–1489.
57. Gasiorek M, Hsieh MH, Forster CS. Utility of DNA next-generation sequencing and expanded quantitative urine culture in diagnosis and management of chronic or persistent lower urinary tract symptoms. *J Clin Microbiol*. 2019;58:e00204–e00219.
58. Price T, Hilt E, Thomas-White K, et al. The urobiome of continent adult women: a cross-sectional study. *BJOG*. 2020;127:193–201.
59. Croxall G, Weston V, Joseph S, et al. Increased human pathogenic potential of *Escherichia coli* from polymicrobial urinary tract infections in comparison to isolates from monomicrobial culture samples. *J Med Microbiol*. 2011;60:102–109.
60. Keogh D, Tay WH, Ho YY, et al. Enterococcal metabolite cues facilitate interspecies niche modulation and polymicrobial infection. *Cell Host Microbe*. 2016;20:493–503.
61. Campbell PM, Humphreys GJ, Summers AM, et al. Does the microbiome affect the outcome of renal transplantation? *Front Cell Infect Microbiol*. 2020;10:558644.
62. Rezatofighi SE, Mirzarazi M, Salehi M. Virulence genes and phylogenetic groups of uropathogenic *Escherichia coli* isolates from patients with urinary tract infection and uninfected control subjects: a case-control study. *BMC Infect Dis*. 2021;21:361.
63. He Q, Hou Q, Wang Y, et al. Comparative genomic analysis of *Enterococcus faecalis*: insights into their environmental adaptations. *BMC Genomics*. 2018;19:527.
64. Wu JF, Muthusamy A, Al-Ghalith GA, et al. Urinary microbiome associated with chronic allograft dysfunction in kidney transplant recipients. *Clin Transplant*. 2018;32:e13436.
65. Patterson JL, Stull-Lane A, Girerd PH, et al. Analysis of adherence, biofilm formation and cytotoxicity suggests a greater virulence potential of *Gardnerella vaginalis* relative to other bacterial-vaginosis-associated anaerobes. *Microbiology (Reading)*. 2010;156:392–399.
66. Lewis WG, Robinson LS, Gilbert NM, et al. Degradation, foraging, and depletion of mucus sialoglycans by the vagina-adapted *Actinobacterium Gardnerella vaginalis*. *J Biol Chem*. 2013;288:12067–12079.
67. Castro J, Alves P, Sousa C, et al. Using an *in-vitro* biofilm model to assess the virulence potential of bacterial vaginosis or non-bacterial vaginosis *Gardnerella vaginalis* isolates. *Sci Rep*. 2015;5:11640.
68. Janulaitiene M, Paliulyte V, Grinceviciene S, et al. Prevalence and distribution of *Gardnerella vaginalis* subgroups in women with and without bacterial vaginosis. *BMC Infect Dis*. 2017;17:394.
69. Gilbert NM, O'Brien VP, Lewis AL. Transient microbiota exposures activate dormant *Escherichia coli* infection in the bladder and drive severe outcomes of recurrent disease. *PLoS Pathog*. 2017;13:e1006238.
70. Shrestha E, White JR, Yu SH, et al. Profiling the urinary microbiome in men with positive versus negative biopsies for prostate cancer. *J Urol*. 2018;199:161–171.
71. Boyanova L, Marteva-Proevska Y, Gergova R, et al. *Gardnerella vaginalis* in urinary tract infections, are men spared? *Anaerobe*. 2021;72:102438.
72. Huang J, Salinas T, Westblade LF, et al. The potential role of the gut microbiota in kidney transplantation. *Kidney360*. 2021;2:890–893.
73. Magruder M, Edusei E, Zhang L, et al. Gut commensal microbiota and decreased risk for *Enterobacteriaceae* bacteriuria and urinary tract infection. *Gut Microbes*. 2020;12:1805281.
74. Magruder M, Sholi AN, Gong C, et al. Gut uropathogen abundance is a risk factor for development of bacteriuria and urinary tract infection. *Nat Commun*. 2019;10:5521.
75. Ahmad S, Bromberg JS. Current status of the microbiome in renal transplantation. *Curr Opin Nephrol Hypertens*. 2016;25:570–576.