

1 **REASSESSMENT OF ROUTINE MIDSTREAM CULTURE IN DIAGNOSIS OF**  
2 **URINARY TRACT INFECTION**

3

4 **Authors:**

5 Sanchutha Sathiananthamoorthy<sup>a,#</sup>, James Malone-Lee<sup>a</sup>, Kiren Gill<sup>a</sup>, Anna Tymon<sup>b</sup>, Trang K.

6 Nguyen<sup>a,\*</sup>, Shradha Gurung<sup>a,\*</sup>, Linda Collins<sup>a,\*</sup>, Anthony S. Kupelian<sup>a,\*</sup>, Sheela Swamy<sup>a</sup>,

7 Rajvinder Khasriya<sup>a,\*</sup>, David A. Spratt<sup>b</sup>, Jennifer L. Rohn<sup>a,#</sup>

8 **Affiliations:**

9 a. Centre for Nephrology, Division of Medicine, University College London, Royal Free  
10 Campus, Rowland Hill Street, London NW3 2PF, UK.

11 b. EDI Department of Microbial Diseases, University College London, 256 Gray's Inn  
12 Road, London WC1X 8LD, UK.

13

14 **Running Head:** Failure of midstream urine culture to detect infection

15

16 **#Address correspondence to:**

17 Sanchutha Sathiananthamoorthy, rebmssa@ucl.ac.uk

18 Jennifer L. Rohn, j.rohn@ucl.ac.uk

19

20 **\*Present addresses:**

21 Trang K Nguyen, University of Leeds, Leeds, UK

22 Shradha Gurung, University of Kent, Kent, UK

23 Linda Collins, Middlesex University, London, UK

24 Anthony S Kupelian, University College Hospital, London, UK

25 Rajvinder Khasriya, University College Hospital, London, UK

26 **ABSTRACT**

27 Midstream urine culture (MSU) remains the gold standard diagnostic test for confirming  
28 urinary tract infection (UTI). We previously showed that patients with chronic lower urinary  
29 tract symptoms (LUTS) below the diagnostic cut-off on MSU culture may still harbour  
30 bacterial infection, and that their antibiotic treatment was associated with symptom  
31 resolution. Here, we evaluated the results of the UK's MSU culture in symptomatic patients  
32 and controls. Next, we compared the bacterial enrichment capabilities of the MSU culture  
33 with a 50 µl uncentrifuged culture, a 30 ml centrifuged sediment culture, and 16S rRNA gene  
34 sequencing. This study was conducted on urine specimens from 33 LUTS patients attending  
35 their first clinical appointment (mean age = 49 years, standard deviation [SD] = 16.5), 30  
36 LUTS patients on treatment (mean age = 47.8 years, SD = 16.8) whose symptoms had  
37 relapsed, and 29 asymptomatic controls (mean age = 40.7 years, SD = 15.7). We showed that  
38 the routine MSU culture, adopting the UK interpretation criteria tailored to acute UTI, failed  
39 to detect a variety of bacterial species, including recognised uropathogens. Moreover, the  
40 diagnostic MSU culture was unable to discriminate between patients and controls. In contrast,  
41 genomic analysis of urine enriched by centrifugation discriminated between the groups,  
42 generating a more accurate understanding of species richness. In conclusion, the UK's MSU  
43 protocol misses a significant proportion of bacteria, which include recognised uropathogens,  
44 and may be unsuitable for excluding UTI in patients with LUTS.

45

46

47

48

49

50

51 **KEY WORDS**

52 Midstream urine culture

53 Urinary tract infection

54 Lower urinary tract symptoms

55 Mixed growth

56 16S rRNA gene sequencing

57

58

59

60

61

62

63

64

65

66

67

68

69

## 70 INTRODUCTION

71 An estimated 150-250 million individuals worldwide develop urinary tract infection (UTI)  
72 every year, making it one of the most frequent types of human infections (1, 2). Midstream  
73 urine culture (MSU) is the gold standard test for its diagnosis. Hospital laboratories in the UK  
74 abide by the Standards for Microbiology Investigations (SMI B 41) protocol, issued and  
75 updated by Public Health England (PHE), which generally recommends a threshold of  $\geq 10^5$   
76 colony forming units per millilitre (cfu/ml) to confirm both uncomplicated and complicated  
77 UTI (3); this criterion is also adopted in many other countries, but varies considerably. The  
78  $10^5$  cfu/ml threshold originates from a comparison conducted in the late 1950s of 74 pregnant  
79 female patients with clinically determined acute pyelonephritis and 337 asymptomatic  
80 women (4, 5).

81 Concerns regarding the suitability of this quantitative cut-off for patients with uncomplicated,  
82 acutely dysuric lower UTI were initially raised by Stamm, for which the evidence-based  
83 suggestion for lowering the colony count to  $10^2$  cfu/ml was concluded to be the more  
84 appropriate alternative (6, 7). It is now recognised that culture, interpreted using a single  
85 quantitative threshold, may not be appropriate when used to detect different clinical  
86 manifestations of UTI (urethritis, cystitis, and pyelonephritis). This may be further  
87 complicated by a multitude of host and microbial factors (8, 9), making it increasingly likely  
88 that no single threshold is suitable for the detection of UTI in every clinical circumstance  
89 (10).

90 The current quantitative MSU threshold is problematic for other reasons. First, bacterial  
91 strains are known to vary in virulence (11), meaning that some strains might generate disease  
92 even at lower concentrations. Second, the concentration of bacteria in urine can vary widely  
93 depending on fluid intake before MSU sampling; a dilute specimen might fall below the

94 diagnostic cut-off as a result. Third, recognised uropathogens, including *Escherichia coli* and  
95 *Enterococcus faecalis*, adhere to host cells and are also known to invade them to form  
96 intracellular colonies (12-14). Thus, urinary supernatant may be a poor substrate for  
97 recovering sequestered organisms. However, it is also known that an innate immune response  
98 to UTI exacerbates the shedding of urothelial cells into urine (15-19).

99 An additional problem associated with standard MSU interpretation in the UK is the  
100 dismissal of mixed growth cultures by default. Conventionally, 'mixed growth' cultures are  
101 assumed to reflect contamination by organisms recognised to colonise the healthy  
102 periurethral, vaginal and perianal regions (5). Although some laboratories electively consider  
103 the growth of up to three organisms each at  $>10^5$  cfu/ml to suggest polymicrobial infection, or  
104 may proceed with a repeat assessment, many disregard mixed cultures altogether (3).

105 However, we and others have shown that polymicrobial infection is common in patients with  
106 lower urinary tract symptoms (LUTS) (13, 20-22). In addition, polymicrobial urosepsis has  
107 been reported in patients who demonstrated identical, mixed isolates from blood and urine  
108 specimens (23, 24). Another study reported that *E. coli*, a predominant uropathogen,  
109 manifested greater invasive properties when isolated from a polymicrobial culture in contrast  
110 to pure growth (25). This finding is supported by recent work showing that *E. faecalis* can  
111 bolster *E. coli* by exporting the nutrient L-ornithine (26).

112 Few studies have analysed the urinary microbial composition by laboratory outcome (i.e.  
113 negative, mixed, positive cultures), and the majority of such work has assessed culture  
114 techniques adopted in US laboratories (6, 27-30). Given the limitations of culture-based  
115 microbial detection (31-33), characterisation of microbial communities in health and disease  
116 is now becoming increasingly dependent on approaches, such as DNA-based identification,  
117 which do not require growth in particular culture conditions (34). Using metagenomics, the  
118 urinary microbiota in patients with neurogenic bladder dysfunction (35), overactive bladder

119 (36), urgency urinary incontinence (UUI) (28), stress urinary incontinence (37) and  
120 uncomplicated UTI (38) have been described. Some studies have also included comparisons  
121 to asymptomatic individuals (28, 33, 35, 36, 39-41).

122 Critical microbiological evaluation of the urine culture protocol adopted in the UK is long  
123 overdue. Here, we chose to study MSU culture performance in patients describing LUTS,  
124 including those who fall short of a positive culture and those demonstrating mixed growth,  
125 since these are generally deemed not to have a *bona fide* infection. This assertion hinges on  
126 standard culture-based diagnosis, and emerging data (13, 28, 42) suggest that such patients  
127 harbour chronic infection. Our aim was to evaluate the MSU culture in symptomatic patients  
128 and asymptomatic controls and assess its performance using both culture and molecular  
129 approaches, with and without specimen enrichment.

130

## 131 **MATERIALS AND METHODS**

132 **Subject recruitment and clinical assessment.** This study obtained ethical approval from the  
133 East London & the City Research Ethics Committee, London, UK. Adult men and women  
134 aged  $\geq 18$  years with LUTS were eligible for study inclusion. Pregnant subjects were not  
135 included in the study. Since urine cultures are relied upon for diagnosis at initial presentation  
136 and during relapse, two separate symptomatic patient groups were recruited and assessed.  
137 Patients attending their first appointment at the Whittington Hospital Lower Urinary Tract  
138 Symptoms (LUTS) Clinic (referred to in this study as “new patients”), who were not on  
139 antibiotic treatment and had not taken antibiotics in the preceding four weeks, were evaluated  
140 by the present clinician and inducted into the first patient group. Patients attending a follow-  
141 up consultation with symptomatic recurrence, relapse or no response to initial antimicrobial  
142 treatment (referred to as “relapsed patients”) were identified following clinical assessment to  
143 form the second patient group. Asymptomatic controls aged  $\geq 18$  years of either sex, with no

144 urological complications and not on antibiotic treatment were recruited from departmental  
145 staff, students and the general population. All patients and controls provided written consent  
146 prior to study inclusion. Figure 1 presents a workflow of all experimental procedures  
147 conducted on each subject. Clinicians overseeing the care of new and relapsed patients were  
148 blinded from all urinalyses results (i.e. urinary dipstick, microscopy and MSU culture test  
149 results). For all downstream analysis, the culture plates were coded with a four-digit study  
150 number and processed separately in large batches. The data for each technique were inputted  
151 separately and comparisons did not take place until all of it had been entered and the code  
152 broken.

153 Symptoms of urgency urinary incontinence (UUI), voiding dysfunction, pain, and stress  
154 urinary incontinence (SUI) were recorded using “yes/no” response questions in a validated  
155 questionnaire (43). The context-related symptoms were summed within each of the four  
156 symptom groups. Patient and control urine specimens were obtained using the clean-catch  
157 MSU method and anonymised with a four-digit study number. Subjects were carefully  
158 instructed in the collection technique. Each urine specimen was analysed using urinary  
159 dipsticks and an automated Clinitek Status analyser (Siemens Healthcare, Germany) for  
160 leukocyte esterase and nitrites. Microscopic leukocyte counts were determined using a  
161 Neubauer counting chamber, loaded with 10  $\mu$ l of fresh urine. Each prepared specimen was  
162 examined by microscopy (x200) using an Olympus CX41 light microscope (Olympus, UK).

163 **National routine diagnostic screening.** An aliquot of each MSU specimen was submitted to  
164 the Whittington Hospital Microbiology Laboratory, London, UK for routine culture. Urine  
165 specimens were stored at 4°C until they were transported to the processing laboratory. These  
166 samples took approximately 60 minutes to arrive and were cultured either immediately on the  
167 same day, or on the next day following overnight storage at 4°C, which reflected usual  
168 practice. The protocol involved inoculating ChromID CPS (now ChromID CPS Elite)

169 chromogenic culture medium (bioMérieux, France) with 1 µl of uncentrifuged urine, which  
170 was then placed in an ordinary incubator at 37°C. Microbial colonies were identified using  
171 the manufacturer's colour criteria. A count of  $\geq 10^5$  cfu/ml for one organism was interpreted  
172 as a significant result. Cultures with a colony count below this threshold were reported as "no  
173 significant growth". MSU cultures, with more than one organism reported as "mixed growth  
174 of *n* types of organisms".

175 **Extended culture-based analysis.** Urine samples were stored at 4°C for 0.5-4.0 hours before  
176 transportation to the research laboratory (University College London, UK), where they were  
177 processed immediately. An identical MSU culture was performed on an aliquot of the same  
178 urine specimen. Simultaneous to this culture, 50 µl of uncentrifuged urine was plated on  
179 chromogenic agar. From the remaining aliquots of each specimen, 30 ml was centrifuged at  
180 1400 x *g* for 10 minutes. The sediment was resuspended in 400 µl of sterilised phosphate  
181 buffer saline (PBS) solution (Life Technologies, UK). Ten-fold serial dilutions were  
182 performed using PBS to reveal the presence of morphologically small-sized colonies, where  
183 the growth of other organisms dominated. All cultures were incubated aerobically at 37°C for  
184 18-24 hours.

185 **Identification of cultured isolates.** Microbial isolates were identified using matrix-assisted  
186 laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) using the  
187 MicroFlex LT mass spectrometer (Bruker Daltonics, USA). In the first instance, the direct  
188 colony plate method was employed for identification and each cultured isolate was inoculated  
189 twice onto the target plate. The target plate was left to air-dry before applying 1 µl of matrix  
190 solution (Bruker Daltonics, USA) consisting of alpha-cyano-4-hydroxycinnamic acid  
191 dissolved in 50% acetonitrile and 2.5% trifluoroacetic acid. The air-dried target plate was  
192 inserted into the mass spectrometer and time was given for the high vacuum to be restored.  
193 Using the MALDI Biotyper 3.0 software programme (Bruker Daltonics, USA), the isolate



194 identifiers were recorded onto the programme and the Bruker Taxonomy library was selected  
195 for the analysis. In the event of an unsuccessful identification, an ethanol-formic acid  
196 extraction protocol was adopted to assist with microbial cell wall disruption and release of  
197 ribosomal proteins (44). A heavy suspension of the unidentified isolate was made in 300 µl of  
198 sterile distilled water. Following the addition of absolute ethanol (900 µl) microbial  
199 suspension, samples were spun down at 20,000 x g for 2 minutes. The resulting ethanol was  
200 discarded and the centrifugation step was repeated. Residual ethanol was removed and the  
201 resulting pellet was left to air-dry at room temperature for 5 minutes. The deposit was  
202 resuspended in 70% formic acid (20 to 50 µl). An equal volume of acetonitrile was added to  
203 this mixture, followed by centrifugation at 20,000 x g for 2 minutes. The supernatant was  
204 then applied onto a sterilised target plate twice and left to air-dry, before resuming with the  
205 direct approach protocol from the point of adding matrix solution to each dried spot.

206 **16S rRNA gene sequencing of urine.** Prior to extraction, urine samples for DNA sequencing  
207 were stored at -80°C. Genomic DNA was extracted from each specimen using an approach  
208 adapted from a previously reported phenol-chloroform-isoamyl alcohol and bead-beating  
209 method (45). Specimens were spun down using a pre-cooled centrifuge at 18,000 x g for 5  
210 minutes at 4°C. Cell pellets were resuspended in extraction buffer (500 µl) consisting of 120  
211 mM potassium phosphate (K<sub>2</sub>PO<sub>4</sub>) buffer (pH 8.0) with 5% cetyltrimethylammonium  
212 bromide (CTAB) (Sigma-Aldrich, USA) in 0.7 M NaCl. The specimen tubes were vortexed  
213 and contents transferred into pre-sterilised 2 ml tubes containing zirconia/silica beads (0.1mm  
214 diameter). Phenol-chloroform-isoamyl (PCI) (25:24:1) alcohol (500 µl) (Invitrogen, USA)  
215 was added to each sample and kept on ice. The samples were homogenised using a RiboLyser  
216 (Hybaid, Germany) for 30 seconds at 5.5 m/s and spun down at 18,000 x g for 15 minutes.  
217 Chloroform-isoamyl (CI) alcohol (Invitrogen, USA) was added at a 1:1 ratio to the extracted  
218 layer of DNA supernatant. After centrifugation at 18,000 x g for 2 minutes at 4°C, the DNA

219 of each sample was precipitated by adding 30% polyethylene glycol (PEG-8000) (Sigma-  
220 Aldrich, USA) at a 2:1 ratio.

221 PCR was performed to amplify the V5-V7 hypervariable regions of the 16S rRNA gene using  
222 the barcoded primers 785F (5'-GGATTAGATACCCBRGTAGTC-3') and 1175R (5'-  
223 ACGTCRTCCCCDCCTTCCTC-3') (see Table S1) (Sigma, UK). Each 25 µl sample reaction  
224 contained 0.125 µl Moltaq DNA polymerase (0.025 µM) (Molzym, VH Bio Ltd, UK), 2.5 µl  
225 of Molzym buffer (x 1) (Molzym, VH Bio Ltd., UK), 0.5 mM MgCl<sub>2</sub>, 0.2 mM dNTPs  
226 (Bioline, UK), 1 µl of forward and reverse primer (0.4 µM) and PCR water (Molzym, VH  
227 Bio Ltd., UK). The PCR reaction stages involved an initial denaturation step at 95°C for 5  
228 minutes and subsequently amplified for 30 cycles at 94°C for 30 seconds (denaturation),  
229 54°C for 40 seconds (annealing), 72°C for 60 seconds (elongation) followed by 72°C for 10  
230 minutes and a final hold of 4°C. All sample reactions were performed in duplex. The DNA  
231 concentrations were determined using the Qubit high-sensitivity DNA assay kit (Life  
232 Technologies, UK) and the Qubit 2.0 fluorometer (Life Technologies, UK). The amplicons  
233 were purified using Agencourt AMPure XP-PCR magnetic particles (Beckman Coulter,  
234 USA) and combined in equimolar ratios using elution buffer (Qiagen, UK) to generate three  
235 pooled DNA libraries (libraries 1, 2 and 3) for pyrosequencing on the MiSeq desktop  
236 sequencer (Illumina Inc., USA), using the v2 reagent kit (Illumina Inc., USA). Since library 3  
237 contained samples with the lowest DNA yields, the purification step was repeated for this  
238 pooled library in an attempt to improve on the final DNA concentration.

239 Our own customised and commercial mock communities were incorporated into DNA  
240 libraries 1 and 2 to verify the DNA sequencing protocol (see Figure S1).

241 **Statistical analysis.** All descriptive and inferential statistics were generated using SPSS  
242 version 25.0 software (IBM, USA). Demographic data, age, gender, 24-hour urinary

243 frequency, nocturia, validated LUTS scores, log-transformed leukocyte counts were  
244 compared across the groups using non-parametric tests. In all cases, except age, homogeneity  
245 of variance was not shown so Mood's median test was used instead of the Kruskal-Wallis.  
246 Hospital MSU culture data (with the outcomes: "negative", "mixed growth" and "positive  
247 culture") were compared across the study groups using the Chi-squared test or Fisher's exact  
248 test.

249 The 16S rRNA data was analysed using QIIME (Quantitative Insights Into Microbial  
250 Ecology, qiime.org version 1.8) (46). The raw reads were demultiplexed and assigned to  
251 samples using barcoded sequences. After demultiplexing, the paired-end data were joined to  
252 obtain a single FASTQ file for each sample. These sequences were then clustered into  
253 Operational Taxonomic Units (OTUs) using an open reference OTU picking strategy. The  
254 OTU clusters were assigned to the Greengenes Reference Database  
255 (<http://greengenes.lbl.gov>) (47) based on 97% sequence similarity (46). OTUs were then  
256 filtered to remove the chimeric sequences (UCHIME) (48) and taxonomy was assigned using  
257 the Ribosomal Database Project (RDP) Classifier (49). Core diversity analyses based on  
258 study group and sample type were performed.

259

## 260 **RESULTS**

261 **Clinical characteristics of the patient cohorts providing samples.** As previous work  
262 suggested that the gold standard MSU culture was not an optimal diagnostic tool to detect  
263 infection in LUTS patients, we set out to directly compare it with two alternative culture  
264 techniques. We collected clean-catch MSU specimens from 33 untreated new patients (mean  
265 age: 49 years, standard deviation [SD] = 16.5) and 30 patients experiencing a symptomatic  
266 relapse (mean age: 47.8 years, SD = 16.8). Since urine cultures are relied upon for diagnosis

267 at initial presentation and during relapse, these two patient groups were recruited and  
268 assessed separately. For comparison, we also recruited 29 asymptomatic controls (mean age:  
269 40.7 years, SD = 15.7). Table 1 details the clinical characteristics of each study cohort.  
270 Statistically similar age distributions were observed for all three study groups ( $\chi^2 = 4.4$ , df =  
271 2,  $P = 0.113$ ). The majority of patients were female, which reflected the well-known  
272 demographics of UTI.

273 Firstly, we inspected the differences between patient and control cohorts. None of the  
274 controls reported any LUTS, thus differing significantly from both patient cohorts studied. A  
275 review of the symptomatic history of new patients ( $n = 30$ , duration of symptoms was not  
276 recorded for three patients) revealed 1 patient (3.0%) to experience symptoms for <1 year, 16  
277 patients (48.5%) for 1-4 years, 9 patients (27.3%) for 5-10 years and 4 patients (12.1%) for  
278  $\geq 10$  years. The median number of daytime and night-time urinary episodes differed across the  
279 three groups, with pairwise post-hoc comparisons identifying a higher median frequency for  
280 new patients and relapsed patients compared with controls (Table 1). We proceeded to  
281 examine the symptomatic differences between new and relapsed patients. Comparisons of  
282 symptom presentation between patient groups revealed that the number of new patients  
283 reporting UUI and SUI symptoms was significantly higher than that of relapsed patients. The  
284 proportion of new and relapsed patients that reported voiding symptoms and pain symptoms  
285 were statistically similar (Table 1).

286 In addition to observing the presence and absence of symptoms, we also calculated a  
287 symptom score to determine the magnitude of LUTS within each patient group. The number  
288 of symptoms was summed for each of the four categories to provide a score and compared  
289 across the groups using Mood's median test. The median symptom scores for UUI, SUI,  
290 voiding and pain symptoms categories differed across the three study groups. Post-hoc  
291 analysis revealed higher median UUI, SUI and voiding scores for new patients than relapsed

292 patients. However, both patient cohorts had similar pain scores and total symptom scores  
293 (Table 1). Statistical comparison of the respective frequencies for each assessed LUTS  
294 between new and relapsed patient groups are provided within the supplementary material  
295 (Table S2).

296 **Urinary microscopy and dipstick analysis.** We performed urinary leukocyte counts to  
297 determine whether patient urine manifested evidence of infection independent of bacterial  
298 assessment. As shown in Table 2, there was a significant difference in  $\log_{10}$  leukocyte counts  
299 among the three study groups ( $\chi^2 = 6.2$ ,  $df = 2$ ,  $P < 0.05$ ). Post-hoc analysis using  
300 Bonferroni's correction identified significant differences between controls and new patients,  
301 and between controls and relapsed patients. No significant difference in  $\log_{10}$  leukocyte  
302 counts was identified between new patients and relapsed patients.

303 Analysis of the urinary dipstick results showed 13 (39.4%) new patients, 11 (36.7%) relapsed  
304 patients and 6 (20.7%) controls tested positive for leukocyte esterase with only 1 (3.0%) new  
305 patient testing positive for urinary nitrite. The results were unsurprising, as previous work  
306 suggested that the dipstick is not sensitive enough to diagnose infection (leukocyte esterase =  
307 46-66%, nitrite = 6-18%), particularly in patients with non-acute symptoms of UTI (50-52).

308 Although LUTS can be driven by non-infectious causes, the association between patient  
309 symptoms and leukocyte recruitment demonstrates urinary tract inflammation. This is critical  
310 if an infective aetiology for LUTS is being considered.

311 **Hospital MSU culture results.** Next, we determined the relationship between patient  
312 symptoms and their standard MSU results. Table 2 summarises the data, in which the  
313 majority of new patients, relapsed patients and controls were reported to have a negative  
314 MSU culture. Six (9.5%) symptomatic patients showed a positive culture result as did two  
315 (6.9%) controls. A comparison of the hospital MSU culture outcomes revealed that the MSU

316 culture was unable to discriminate between the three study groups ( $\chi^2 = 1.7$ ,  $df = 4$ ,  $P =$   
317  $0.787$ ). The hospital reports showed that all four new patients with positive cultures grew *E.*  
318 *coli*, whereas *Enterococcus* ( $n = 1$ ) and a coliform belonging to the  
319 *Klebsiella/Enterobacter/Serratia* group ( $n = 1$ ) were reported for the positive MSU cultures  
320 of relapse patients. *Proteus* ( $n = 1$ ) and *Streptococcus agalactiae* ( $n = 1$ ) were cultivated from  
321 controls.

322 Further pairwise statistical comparisons of the number of positive and negative MSU cultures  
323 using the Fisher's exact test were performed. For this particular analysis, "mixed growth" and  
324 "no significant growth" cultures were under the category of "negative" as both results are  
325 traditionally dismissed as insignificant. This analysis indicated that even with the exclusion  
326 of the relapsed group, the MSU culture was still unable to discriminate between  
327 asymptomatic controls and new patients ( $\chi^2 = 0.539$ ,  $df = 1$ ,  $P = 0.674$ ). The same analysis for  
328 relapsed patients and controls also showed no significant difference ( $\chi^2 = 0.0$ ,  $df = 1$ ,  $P =$   
329  $1.0$ ). Likewise, comparison of new patients and relapsed patients showed no difference ( $\chi^2 =$   
330  $0.539$ ,  $df = 1$ ,  $P = 0.674$ ). These results further highlight the inability of the MSU culture to  
331 discriminate between the three study groups.

332 Since the hospital laboratory did not report the microbial composites of mixed growth and  
333 negative MSU cultures, we inspected the 1  $\mu$ l loop MSU cultures that were replicated in our  
334 own research laboratories subjecting colonies grown on chromogenic agar to MALDI-TOF  
335 MS analysis for identification. The pooled percentage frequencies of organisms identified  
336 from the polymicrobial MSU cultures of new patients ( $n = 7$ ), relapsed patients ( $n = 4$ ) and  
337 controls ( $n = 5$ ) are shown in Figure S2. No colonies on the research laboratory MSU culture  
338 were observed for 17 (58.6%) controls, 12 (36.4%) new patients, and 13 (43.3%) relapsed  
339 patients. Whilst 19 isolates were grown from 12 asymptomatic control MSU cultures, 39  
340 isolates were identified from 21 new patient cultures and 25 isolates were grown from 17

341 relapsed patient cultures. With this replicated diagnostic culture, *Enterococcus* and  
342 *Escherichia* were predominantly cultured from new patients and relapsed patients, whereas  
343 *Streptococcus* and *Staphylococcus* were most frequently isolated from controls. Within this  
344 sample size, Fisher's exact test confirmed a significantly higher frequency of *Escherichia* ( $\chi^2$   
345 = 5.4, df = 1,  $P < 0.05$ ) and *Enterococcus* ( $\chi^2 = 8.9$ , df = 1,  $P < 0.05$ ) isolated from new  
346 patient cultures than from controls. The relapsing patient group was also observed to produce  
347 a significantly higher number of *Enterococcus* isolates than the control group ( $\chi^2 = 6.4$ , df =  
348 1,  $P < 0.05$ ). In summary, the quantitative MSU had no discriminatory diagnostic power, but  
349 the microbial communities isolated in the process showed some differences between patients  
350 and controls.

351 **Comparison of culture techniques.** We compared the discriminatory power of the hospital  
352 MSU culture with other methods, specifically plating a larger volume of uncentrifuged urine,  
353 and plating samples enriched by centrifugation. As shown in Figure 2, microbial growth was  
354 categorised as “no growth”, “one isolate” and “ $\geq$  two isolates” for comparison. Of note, as the  
355 amount of bacterial input increased (from 1  $\mu$ l supernatant to 50  $\mu$ l supernatant to centrifuged  
356 sediment); regardless of patient group, more polymicrobial growths were revealed. Using this  
357 alternative categorisation, the MSU culture replicated within our research laboratory was still  
358 unable to discriminate between the three study groups ( $\chi^2 = 4.6$ , df = 4,  $P = 0.326$ ).  
359 Additionally, culturing more supernatant (50  $\mu$ l) or the sediment did not improve  
360 differentiation across the three cohorts ( $\chi^2 = 11.7$ , df = 4,  $P = 0.02$  [no significant differences  
361 identified with post-hoc analysis] and  $\chi^2 = 8.4$ , df = 4,  $P = 0.078$ . respectively), likely because  
362 as we and others have reported, healthy bladders also harbour polymicrobial growths. These  
363 results suggest that quantitative microbiology is not an adequate diagnostic tool for patients  
364 experiencing LUTS.

365 **Patient and control urinary bacterial communities.** Figures 3 illustrates pairwise study  
366 group comparisons of the genera and respective percentage frequencies identified on the  
367 MSU culture (reproduced in-house), 50  $\mu$ l urine unspun culture and 30 ml sediment cultures  
368 (See figure S3 for species-level characterisation across study groups). As seen with the MSU  
369 culture results, differences were noted among the various groups on sediment culture. Whilst  
370 15 different genera were grown from symptomatic patient sediment cultures ( $n = 13$  from  
371 new patients,  $n = 11$  from relapsed patients) eight genera were isolated from control sediment  
372 cultures. Seven genera were shared by both patient and control groups, which were  
373 *Corynebacterium*, *Enterococcus*, *Escherichia*, *Klebsiella*, *Proteus*, *Staphylococcus* and  
374 *Streptococcus*. Organisms that were cultivated from patient samples that were not isolated  
375 from control specimens were *Candida*, *Citrobacter*, *Enterobacter*, *Lactobacillus*, *Leclercia*,  
376 *Morganella* and *Pseudomonas*. In both symptomatic patient groups, *Enterococcus*,  
377 *Staphylococcus* and *Escherichia* were the most abundant organisms. In asymptomatic  
378 controls, *Staphylococcus*, *Enterococcus* and, *Streptococcus* were most frequently isolated.  
379 However, *Staphylococcus* ( $\chi^2 = 3.7$ ,  $df = 1$ ,  $P = 0.064$ ) and *Streptococcus* ( $\chi^2 = 2.4$ ,  $df = 1$ ,  $P$   
380  $= 0.2$ ) were present at statistically similar frequencies in patients. Fisher's exact test revealed  
381 a significantly higher frequency of *Enterococcus* isolates cultivated from the new patients  
382 than from asymptomatic controls ( $\chi^2 = 6.2$ ,  $df = 1$ ,  $P < 0.05$ ). No significant difference was  
383 observed with any other genus between new patients and controls. Comparison of frequencies  
384 between control and relapsed patient groups using Fisher's exact test revealed a significantly  
385 higher frequency of *Staphylococcus* ( $\chi^2 = 6.9$ ,  $df = 1$ ,  $P < 0.05$ ), and *Streptococcus* ( $\chi^2 = 7.0$ ,  
386  $df = 1$ ,  $P < 0.05$ ) in controls than relapsed patients. No significant differences in the number  
387 of genera were identified between new and relapsed patient groups.

388 We went on to analyse urinary bacteria in unprocessed urine (1 ml uncentrifuged) versus 30  
389 ml of urine enriched by centrifugation in new patient versus control study groups using next-



390 generation sequencing based on rDNA. The percentage of identified sequencing reads or  
391 reads that passed filter (PF) (Q30 >70%) for loaded DNA libraries 1, 2 and 3 were: (i) 77.9%  
392 (10,122,606 PF reads), (ii) 80.0% (14,910,030 PF reads) and (iii) 68.3% (10,129,563 PF  
393 reads) respectively. Bacterial DNA was detected in the urine samples of 32 (97.0%) of 33  
394 recruited new patients (30 uncentrifuged urine samples and 30 centrifuged samples), 26  
395 (89.7%) of 29 control samples (22 uncentrifuged samples and 23 centrifuged samples).  
396 Samples that yielded low DNA concentrations were salvaged with an extended protocol.  
397 Beyond this, bacterial DNA was undetectable in one new patient and three controls.

398 Figure 4 presents the relative abundance of the 20 most abundant taxa identified from the  
399 uncentrifuged and centrifuged urine samples of new patients and controls (see supplementary  
400 Table S3 for hierarchical classifications). The 20 most abundant taxa made up 81.0% of new  
401 patient sequences and 79.0% of control sequences. The most abundant taxa of the  
402 uncentrifuged urinary bacterial community of new patients from highest to lowest were  
403 *Enterobacteriaceae* (32.3%), followed by *Lactobacillus* (15.5%) and *Streptococcus* (8.7%)  
404 and *Enterococcus* (8.0%), whereas the most abundant taxa of the new patient urinary  
405 microbial community represented by centrifuged samples were *Enterobacteriaceae* (26.9%),  
406 *Enterococcus* (12.8%), *Psychrobacter* (9.3%) and *Streptococcus* (8.3%). The most abundant  
407 taxa identified from uncentrifuged control samples were *Streptococcus* (21.5%),  
408 *Enterobacteriaceae* (20.1%), *Lactobacillus* (11.6%) and *Gardnerella* (7.5%). In contrast, the  
409 most abundant taxa identified from centrifuged control urine samples were *Streptococcus*  
410 (15.8%), *Staphylococcus* (14.8%), *Enterobacteriaceae* (11.5%) and *Lactobacillus* (9.2%).  
411 From these data, a decrease in overall abundance of *Lactobacillus* (15.5% uncentrifuged,  
412 7.8% centrifuged) and an increase in abundance of *Enterococcus* (8.0% uncentrifuged, 12.8%  
413 centrifuged) were observed with the new patient centrifuged urinary community when  
414 compared with the new patient uncentrifuged community. On the other hand, the control

415 centrifuged community showed an increase in overall abundance of *Staphylococcus* (1.9%  
416 uncentrifuged, 14.8% centrifuged) compared with the control uncentrifuged urinary  
417 community. Centrifugation of samples therefore has a strong influence on species recovery.  
418 Moreover, this approach also highlights differences in microbial composition between  
419 patients and controls (similar to what was seen in our sediment culture data [Figure 3]).

420 Table 3 presents the richness and diversity measures of new patient and control urinary tract  
421 bacterial communities. Richness was assessed by comparing the mean number of OTUs and  
422 mean Chao1 estimator values using the Welch's two-sample *t* test. Diversity was assessed by  
423 comparing the mean Shannon index and mean inverse Simpson's index using the Welch's  
424 two-sample *t* test. Pairwise comparisons indicated no significant difference in the mean  
425 number of observed OTUs and Chao1 estimator between patient and control uncentrifuged  
426 samples, centrifuged samples and both combined. Additionally, no significant difference was  
427 observed in the mean Shannon's index and inverse Simpson's index between patients and  
428 control communities.

429 Overall, our analyses show a clear difference between patients with LUTS and controls,  
430 namely that the *Enterobacteriaceae* was the most abundant taxa associated with disease, and  
431 *Streptococcus*, with health. However, it is clear the method of sample processing enriches for  
432 different taxa. Specifically, centrifugation enriched *Enterococcus* in patient samples, and  
433 *Staphylococcus* in controls.

434 **Comparison of sediment culture and sequencing approaches.** As shown in Figure 5,  
435 colour-coded matrices consisting of all cultured bacterial taxa that were detected by  
436 centrifuged sediment culture and 16S rRNA gene sequencing were generated for new patient  
437 centrifuged samples and control centrifuged urine samples. Among all centrifuged samples  
438 37 (59.7%) of 62 contained bacteria that were detected by both culture and sequencing

439 methods. Three (9.1%) patient and four (13.8%) control centrifuged samples were sequence-  
440 negative, but culture-positive. Two (6.1%) patient and three (10.3%) centrifuged control  
441 samples were culture-negative, but sequence-positive *Enterococcus* was the most frequently  
442 identified bacterium from both sediment culture and sequencing methods. These genera were  
443 identified from 22 (66.7%) new patient samples. *Staphylococcus* was the most frequently  
444 identified bacterium by both methods from control samples. This genus was identified from  
445 11 (37.9%) centrifuged samples. *Escherichia* demonstrated the most cases ( $n = 14$ ) in which  
446 it was detected by sediment culture, but remained undetected by sequencing. One new patient  
447 sample (NL5) grew *Leclercia* on sediment culture, but this particular taxa was not detected  
448 by sequencing. This analysis shows that the sequencing method is vastly superior to culture  
449 techniques for bacterial detection, and that culture methods do not recover a large number of  
450 bacterial species, including recognised uropathogens.

451

## 452 **DISCUSSION**

453 A recent commentary in *J Clin Micro* emphasised how the diagnostic landscape for UTI is  
454 becoming increasingly challenging in the face of alternatives to standard culture (53). The  
455 primary purpose of this study was to evaluate the routine MSU culture performed in UK  
456 diagnostic laboratories. This evaluation is crucial, since culture is considered the gold  
457 standard diagnostic test for confirming or excluding UTI in symptomatic patients, although  
458 evidence suggests that it may be deficient (6, 7). In the UK, symptom-based empirical  
459 antibiotic treatment for acute UTI is recommended (54). The evidence suggests that the  
460 diagnostic accuracy of acute symptoms in previously asymptomatic subjects outperforms  
461 routine culture-based diagnosis (55). By contrast, relapsing patients with LUTS are not as  
462 straightforward and may demonstrate a distinct microbiota under the influence of prescribed

463 antimicrobials. The cohort of relapsing patients was consequently excluded from genomic  
464 analysis to aid in clarifying the differences in urinary microbial communities between first-  
465 visit patients with LUTS and health. Despite testing negative on routine MSU, such patients  
466 have been shown to harbour uropathogens when their urine is examined with more sensitive  
467 methods (13, 28, 29, 36, 42). Furthermore, symptomatic resolution has been observed  
468 following antibiotic treatment in this type of patient (56). Therefore, we questioned whether  
469 current routine MSU cultures with a  $>10^5$  cfu/ml threshold were sensitive enough to detect  
470 UTI in patients presenting with LUTS.

471 Following a symptomatic assessment of LUTS patients, we scrutinised the microbial  
472 composition of each patient and control MSU culture and compared these to the results  
473 reported by the hospital diagnostic laboratory, as well as to organisms identified using other  
474 alternative culture and molecular methods. Crucially, we found that the routine MSU culture  
475 results reported by the diagnostic laboratory failed to discriminate between patients and  
476 control groups. Fewer than 10% of patients with a clinically suspected UTI had a positive  
477 MSU culture, with only four of 33 new patient cultures producing monomicrobial growth at  
478  $10^5$  cfu/ml. In contrast, bacterial DNA sequencing revealed bacteria including recognised  
479 uropathogens in 30 of 32 new patients, including symptomatic patients who were reported to  
480 have a negative MSU culture. In this study, 16S rRNA gene sequencing was not applied as a  
481 diagnostic test, but rather, it was used to determine whether recognised uropathogens were  
482 missed with routine culture techniques, and to characterise symptomatic and healthy urinary  
483 bacterial communities.

484 Our second key finding was that MSU culture grossly underestimated the amount of  
485 polymicrobial growth in patient urine. For example, 36.4% of new patient urines grew mixed  
486 growths; this number rose to 63.6% when more uncentrifuged urine was plated, and to 84.8%  
487 in the case of centrifuged sediment cultures. Although it is now recognised that even healthy

488 urine contains bacteria (41), which certainly complicates the diagnostic landscape, our  
489 enhanced culture and genomic analyses showed a clear difference between the taxa isolated  
490 between patients and controls. Specifically, *Enterobacteriaceae* was the dominant family in  
491 new patients, whereas *Streptococcus* was foremost in asymptomatic controls. These findings,  
492 taken together with other reports, suggest that automatically discounting polymicrobial  
493 cultures may dismiss symptomatic patients in need of treatment.

494 There is of course a formal possibility that the mixed growths reported here could simply be  
495 the result of contamination by skin bacteria during sample collection. In the UK, this  
496 possibility is addressed by the UK Standards for Microbiology Investigations, which  
497 recommends the counting of squamous epithelial cells in the urine as a marker for  
498 contamination. However, their utility for this purpose is complicated by the fact that  
499 exfoliation of urothelial cells as an innate immune response to UTI is a recognised  
500 phenomenon, which is widely used as a surrogate marker of infection in both mice and  
501 humans (14, 21, 57-65). Indeed, using antibodies against uroplakin III, a highly specific  
502 marker for cells originating from the urinary tract, we previously demonstrated that the  
503 majority of epithelial cells shed in the urine of symptomatic patients originate from the  
504 bladder (14). Finally, were the presence of mixed growths merely the result of contamination,  
505 we would not expect their presence to cluster statistically with symptoms as they have done  
506 in this study.

507 Of note, some UTI-associated organisms were shared at the culture level by symptomatic and  
508 asymptomatic patients: *Corynebacterium*, *Enterococcus*, *Escherichia*, *Klebsiella*, *Proteus*,  
509 *Staphylococcus* and *Streptococcus*. The presence of “uropathogenic” species in controls could  
510 reflect a lack of the appropriate virulence factor expression (11), or immune differences in the  
511 host (for e.g. (66)). However, similar community profiles in the bladder have been seen by  
512 others (13, 28, 29, 42, 67), which makes it likely that the ability to be pathogenic is highly

513 context-dependent, and could therefore be influenced by the presence of other species. Such  
514 pathobiont relationships are common in the microbial world (68) and in the case of UTI, have  
515 been reported in mixed infections with *E. coli* and *Enterococcus* (25, 26). Furthermore,  
516 voided urine passes through the urethra as well as the bladder; the microbial ecology of the  
517 urethra is poorly understood in the case of UTI (69-71), and further studies are needed to  
518 understand what role such urethral communities might play.

519 Further afield, microbial community inhabitants in adjacent niches such as the vagina and  
520 perianal region may also influence what species induce UTI pathophysiology. For example,  
521 recent work by Gilbert *et al.* showed that transient exposure to the vaginal organism  
522 *Gardnerella vaginalis* could induce dysbiosis, reactivating dormant *E. coli* in previously  
523 infected mouse bladders (64). Other interactions could be protective; for example, the vaginal  
524 commensal *Lactobacillus crispatus*, which is known to make the vagina less hospitable to  
525 certain Gram-negative perianal uropathogens, may decrease the instance of recurrent UTI  
526 (72, 73). Indeed, our results showing that *Lactobacillus* is a dominant organism among the  
527 control samples, consistent with reports from other groups comparing the urine of patients  
528 with LUTS with asymptomatic controls, supports this notion (28). Further research, including  
529 detailed sequence comparisons, is required to understand the mobility and interspecies effects  
530 of the various microbial inhabitants in the region perianal and urethra region. This could  
531 further our understanding of the significance of polymicrobial communities in UTI.

532 Of interest, the process of centrifugation had a major effect on which taxa dominated,  
533 enriching *Enterococcus* in patient samples, and *Staphylococcus* in controls. This is perhaps  
534 unsurprising, given that uropathogens are known to strongly adhere to and, in some cases,  
535 invade urothelial cells (14). These cells are shed into the urine via an innate immune response  
536 that is yet to be fully characterised (63, 74). Therefore, a significant proportion of bacteria in  
537 such specimens – and perhaps those most relevant for pathophysiology – could be cell-

538 associated, not planktonic. The MSU culture samples a small amount of urine supernatant  
539 and does not access the cell-rich sediment that settles by gravity. Several reports from the  
540 Brubaker group also recognised the limitations of standard culture and developed the  
541 expanded quantitative urine culture (EQUC) protocol. This involves inoculating  
542 uncentrifuged urine onto a broader selection of culture media for optimal species isolation  
543 (28, 36, 42). The approach demonstrated that the routinely performed standard quantitative  
544 culture missed 67% of recognised uropathogens, with the authors suggesting that the method  
545 be used to supplement the standard culture (42). They did not however analyse centrifuged  
546 sediment with this protocol.

547 In addition to factors beyond control, such as the volume of fluid intake before urine  
548 sampling, even the use of a broader selection of culture media has its limitations (31). The  
549 cultured urinary community depends on the types of specimens analysed, techniques  
550 employed, nutrient medium used, laboratory conditions (e.g. pH, temperature, O<sub>2</sub>  
551 concentration), as well as the viability, facultative and fastidious natures of urinary tract  
552 organism. Such factors can distort the species richness of the urinary tract community.  
553 Additionally, bacteria may exist in the viable but not cultivable (VBNC) state, also referred to  
554 as conditionally viable environmental (CVEC) cells, active but not cultivable (ABNC) or  
555 dormant cells (75). Such species are more reliably represented by nucleic acid-based  
556 approaches.

557 In their studies, Brubaker *et al.* examined bladder urine specimens (specifically collected by a  
558 transurethral catheter [CSU] or suprapubic aspiration [SPA]) (28, 33, 36, 42). In our study,  
559 we used a clean-catch MSU specimen collection technique for several reasons. The aim of  
560 this study was to evaluate the routine MSU culture technique, which is the primary method in  
561 the clinic for collecting a urine sample for culture. In symptomatic patients, catheterisation is  
562 potentially an invasive, uncomfortable and painful method of collection that has also been

563 reported to increase the risk of developing an infection (76). Furthermore, catheterisation may  
564 bypass organisms colonising the urethra, which may represent an early stage UTI or  
565 contribute to the infection. Hooton *et al.* discussed this as a possible reason for why a lower  
566 threshold is usually applied to CSU cultures and a higher count is considered for MSU  
567 cultures, since it is unclear if the latter collection method has sampled bacteria from the  
568 bladder and/or the urethral region (27).

569 Female subjects formed the majority of patients and controls, in line with the demographics  
570 of UTI. Given that a previous study found some differences between the microbial ecology in  
571 male and female bladders (77), it would be interesting to further explore the effect of sex on  
572 symptomatic urinary microbiota.

573 The lack of statistical difference between patient and control uncentrifuged samples was  
574 consistent with other studies (28, 78) and could be attributed to the small study cohort.  
575 However statistical comparison of all patient samples (uncentrifuged and centrifuged) to all  
576 control samples (uncentrifuged and centrifuged) using the non-parametric Kruskal-Wallis test  
577 did confirm a significantly higher median number of OTUs in patients than controls. This  
578 suggests that incorporating centrifuged urine samples may clarify the distinction between  
579 patient and control bacterial community richness.

580 While region-specific 16S rRNA gene sequencing using the MiSeq platform provided a less  
581 biased representation of the urinary bacterial community, it is important to acknowledge the  
582 limitations of this method. Due to the short-read sequencing approach used, approximately  
583 30% (approximately 500 nucleotides) of the 16S rRNA gene was amplified, which makes  
584 identification at the genus level possible, but reliable taxonomic assignment of reads at the  
585 species level elusive. Our comparison of taxa identified by culture and 16S rRNA gene  
586 sequencing revealed that the sequencing method was more capable of identifying the



587 cultivable bacterial taxa from patient and control samples compared with the centrifuged  
588 sediment culture technique. However, the sediment culture method permitted bacterial  
589 identification at the species level, which could not be achieved reliably by 16S rRNA gene  
590 sequencing directly from urine. Of note, *Escherichia* demonstrated one the highest rates for  
591 detection by culture but was often undetected by DNA sequencing among centrifuged and  
592 uncentrifuged samples. This genus belongs to the *Enterobacteriaceae* family, which are  
593 reported to be challenging to identify reliably at the genus level due to the high degree of  
594 sequence similarities (79). Given that *E. coli* is a prominent uropathogen, improvements are  
595 needed. A molecular species-level characterisation could be achieved using the more recently  
596 developed third generation sequencing technologies, including the MinION platform series  
597 (80). The potential to reconstruct more than 90% of the 16S rRNA gene makes species-level  
598 characterisation possible.

599 This study underlines the potential weaknesses of the MSU culture for diagnosing UTI in  
600 patients affected by LUTS, which should now be assessed further in a larger, multi-centre  
601 study. These patients appear to harbour chronic infection, missed by quantitative cultures and  
602 most effectively demonstrated by non-culture techniques. These methods shed light on the  
603 complexity of the bacterial communities within these patients, whose symptoms and the  
604 urinary white blood cell count may be the best indicators of infection until the disease  
605 landscape is better understood. In the meantime, those responsible for UTI detection,  
606 diagnosis and patient care, including clinicians and microbiologists, may wish to use caution  
607 when interpreting a negative or mixed growth MSU result in symptomatic patients, as well as  
608 reassess reporting and treatment guidelines to arrive at a solution most appropriate for  
609 patients, in a way that also preserves antibiotic stewardship efforts (53). Ultimately, a point-  
610 of-care test based on a sound understanding of the relevant microbiomes using emerging

611 portable genomic technology would revolutionise the diagnostic landscape for this common  
612 affliction.

613

#### 614 **ACKNOWLEDGEMENTS**

615 We would like to thank UCL Crucible Research Programme for funding this work. The  
616 funders were not involved in the study design, data collection, interpretation or decision to  
617 submit work for publication.

618 We thank Neil Jones and Poonam Sanganee (Microbiology Department, Whittington  
619 Hospital, UK) for their technical support with MALDI-TOF MS-based microbial  
620 identification and Tony Brooks (Genomics Centre, University College London, UK) for his  
621 assistance with library denaturation and final preparation stages of the 16S rRNA gene  
622 sequencing work. We thank members of the EDI Microbial Diseases laboratory (University  
623 College London, UK) for their advice on DNA library preparation. We also thank Sanjay  
624 Khadayate (MRC London Institute of Medical Sciences, Imperial College London, UK) for his  
625 advice and assistance with the bioinformatics analysis.

626 We are very grateful to Sophie Collier and Verve Enne for critically reviewing the  
627 manuscript.

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

631 J. R. has received funding from AtoCap Ltd.

632

633 **REFERENCES**

- 634 1. Harding GKM, Ronald AR. 1994. The management of urinary infections; what have  
635 we learned in the past decade? *International Journal of Antimicrobial Agents* 4:83-88.
- 636 2. Flores-Mireles AL, Walker JN, Caparon M, Hultgren SJ. 2015. Urinary tract  
637 infections: epidemiology, mechanisms of infection and treatment options. *Nat Rev*  
638 *Micro* 13:269-284.
- 639 3. Public Health England. 2018. SMI B41: UK Standards for Microbiology  
640 Investigations-Investigation of urine. United Kingdom.  
641 <https://www.gov.uk/government/publications/smi-b-41-investigation-of-urine>.  
642 Accessed September 2018.
- 643 4. Kass EH. 1956. Asymptomatic infections of the urinary tract. *Trans Assoc Am*  
644 *Physicians* 69:56-64.
- 645 5. Kass EH. 1957. Bacteriuria and the diagnosis of infections of the urinary tract; with  
646 observations on the use of methionine as a urinary antiseptic. *AMA Arch Intern Med*  
647 100:709-14.
- 648 6. Stamm WE, Counts GW, Running KR, Fihn S, Turck M, Holmes KK. 1982.  
649 Diagnosis of coliform infection in acutely dysuric women. *N Engl J Med* 307:463-8.
- 650 7. Stamm WE. 1983. Interpretation of urine cultures. *Clinical Microbiology Newsletter*  
651 5:15-17.
- 652 8. Köves B, Wullt B. 2016. The Roles of the Host and the Pathogens in Urinary Tract  
653 Infections. *European Urology Supplements* 15:88-94.
- 654 9. Stamm WE. 2006. Theodore E. Woodward Award: Host-Pathogen Interactions in  
655 Community-Acquired Urinary Tract Infections. *Transactions of the American Clinical*  
656 *and Climatological Association* 117:75-84.

- 657 10. Grabe M, Bjerklund-Johansen TE, Botto H, Wullt B, Çek M, Naber KG, Pickard RS,  
658 Tenke P, Wagenlehner F. 2011. Guidelines on Urological Infections.  
659 [http://www.uroweb.org/gls/pdf/15\\_Urological\\_Infections.pdf](http://www.uroweb.org/gls/pdf/15_Urological_Infections.pdf). Accessed December  
660 2012.
- 661 11. Wiles TJ, Kulesus RR, Mulvey MA. 2008. Origins and Virulence Mechanisms of  
662 Uropathogenic *Escherichia coli*. *Experimental and molecular pathology* 85:11-19.
- 663 12. Hunstad DA, Justice SS. 2010. Intracellular lifestyles and immune evasion strategies  
664 of uropathogenic *Escherichia coli*. *Annu Rev Microbiol* 64:203-21.
- 665 13. Khasriya R, Sathiananthamoorthy S, Ismail S, Kelsey M, Wilson M, Rohn JL,  
666 Malone-Lee J. 2013. Spectrum of bacterial colonization associated with urothelial  
667 cells from patients with chronic lower urinary tract symptoms. *J Clin Microbiol*  
668 51:2054-62.
- 669 14. Horsley H, Malone-Lee J, Holland D, Tuz M, Hibbert A, Kelsey M, Kupelian A,  
670 Rohn JL. 2013. *Enterococcus faecalis* Subverts and Invades the Host Urothelium in  
671 Patients with Chronic Urinary Tract Infection. *PLoS One* 8:e83637.
- 672 15. Mulvey MA, Lopez-Boado YS, Wilson CL, Roth R, Parks WC, Heuser J, Hultgren  
673 SJ. 1998. Induction and evasion of host defenses by type 1-piliated uropathogenic  
674 *Escherichia coli*. *Science* 282:1494-7.
- 675 16. Mulvey MA, Schilling JD, Hultgren SJ. 2001. Establishment of a persistent  
676 *Escherichia coli* reservoir during the acute phase of a bladder infection. *Infect Immun*  
677 69:4572-9.
- 678 17. Elliott TS, Reed L, Slack RC, Bishop MC. 1985. Bacteriology and ultrastructure of  
679 the bladder in patients with urinary tract infections. *J Infect* 11:191-9.
- 680 18. Mysorekar IU, Isaacson-Schmid M, Walker JN, Mills JC, Hultgren SJ. 2009. Bone  
681 morphogenetic protein 4 signaling regulates epithelial renewal in the urinary tract in  
682 response to uropathogenic infection. *Cell host & microbe* 5:463-475.

- 683 19. Hayes BW, Abraham SN. 2016. Innate Immune Responses to Bladder Infection.  
684 Microbiology Spectrum 4.
- 685 20. Kline KA. 2016. Mechanisms of enterococcal niche modulation during polymicrobial  
686 infection, vol 118, 638. National Institute of Health, Nanyang Technological  
687 University, Singapore.
- 688 21. Gill K, Kang R, Sathiananthamoorthy S, Khasriya R, Malone-Lee J. 2018. A blinded  
689 observational cohort study of the microbiological ecology associated with pyuria and  
690 overactive bladder symptoms. International Urogynecology Journal  
691 doi:10.1007/s00192-018-3558-x.
- 692 22. de Vos MGJ, Zagorski M, McNally A, Bollenbach T. 2017. Interaction networks,  
693 ecological stability, and collective antibiotic tolerance in polymicrobial infections.  
694 Proc Natl Acad Sci U S A 114:10666-10671.
- 695 23. Siegman-Igra Y. 1994. The significance of urine culture with mixed flora. Curr Opin  
696 Nephrol Hypertens 3:656-9.
- 697 24. Siegman-Igra Y, Kulka T, Schwartz D, Konforti N. 1993. The significance of  
698 polymicrobial growth in urine: contamination or true infection. Scand J Infect Dis  
699 25:85-91.
- 700 25. Croxall G, Weston V, Joseph S, Manning G, Cheetham P, McNally A. 2011.  
701 Increased human pathogenic potential of Escherichia coli from polymicrobial urinary  
702 tract infections in comparison to isolates from monomicrobial culture samples. J Med  
703 Microbiol 60:102-9.
- 704 26. Keogh D, Tay WH, Ho YY, Dale JL, Chen S, Umashankar S, Williams RB, Chen SL,  
705 Dunny GM, Kline KA. 2016. Enterococcal Metabolite Cues Facilitate Interspecies  
706 Niche Modulation and Polymicrobial Infection. Cell Host Microbe 20:493-503.

- 707 27. Hooton TM, Roberts PL, Cox ME, Stapleton AE. 2013. Voided Midstream Urine  
708 Culture and Acute Cystitis in Premenopausal Women. *New England Journal of*  
709 *Medicine* 369:1883-1891.
- 710 28. Pearce MM, Hilt EE, Rosenfeld AB, Zilliox MJ, Thomas-White K, Fok C,  
711 Kliethermes S, Schreckenberger PC, Brubaker L, Gai X, Wolfe AJ. 2014. The Female  
712 Urinary Microbiome: a Comparison of Women with and without Urgency Urinary  
713 Incontinence. *mBio* 5.
- 714 29. Coorevits L, Heytens S, Boelens J, Claeys G. 2017. The resident microflora of voided  
715 midstream urine of healthy controls: standard versus expanded urine culture  
716 protocols. *Eur J Clin Microbiol Infect Dis* 36:635-639.
- 717 30. Price TK, Hilt EE, Dune TJ, Mueller ER, Wolfe AJ, Brubaker L. 2018. Urine trouble:  
718 should we think differently about UTI? *International Urogynecology Journal* 29:205-  
719 210.
- 720 31. Spratt DA. 2004. Significance of bacterial identification by molecular biology  
721 methods. *Endodontic Topics* 9:5-14.
- 722 32. Rappe MS, Giovannoni SJ. 2003. The uncultured microbial majority. *Annu Rev*  
723 *Microbiol* 57:369-94.
- 724 33. Wolfe AJ, Toh E, Shibata N, Rong R, Kenton K, Fitzgerald M, Mueller ER,  
725 Schreckenberger P, Dong Q, Nelson DE, Brubaker L. 2012. Evidence of uncultivated  
726 bacteria in the adult female bladder. *J Clin Microbiol* 50:1376-83.
- 727 34. The Human Microbiome Project C, Huttenhower C, Gevers D, Knight R, Abubucker  
728 S, Badger JH, Chinwalla AT, Creasy HH, Earl AM, FitzGerald MG, Fulton RS,  
729 Giglio MG, Hallsworth-Pepin K, Lobos EA, Madupu R, Magrini V, Martin JC,  
730 Mitreva M, Muzny DM, Sodergren EJ, Versalovic J, Wollam AM, Worley KC,  
731 Wortman JR, Young SK, Zeng Q, Aagaard KM, Abolude OO, Allen-Vercoe E, Alm  
732 EJ, Alvarado L, Andersen GL, Anderson S, Appelbaum E, Arachchi HM, Armitage

733 G, Arze CA, Ayvaz T, Baker CC, Begg L, Belachew T, Bhonagiri V, Bihan M, Blaser  
734 MJ, Bloom T, Bonazzi V, Paul Brooks J, Buck GA, Buhay CJ, Busam DA, et al.  
735 2012. Structure, function and diversity of the healthy human microbiome. *Nature*  
736 486:207.

737 35. Fouts DE, Pieper R, Szpakowski S, Pohl H, Knoblach S, Suh MJ, Huang ST,  
738 Ljungberg I, Sprague BM, Lucas SK, Torralba M, Nelson KE, Groah SL. 2012.  
739 Integrated next-generation sequencing of 16S rDNA and metaproteomics differentiate  
740 the healthy urine microbiome from asymptomatic bacteriuria in neuropathic bladder  
741 associated with spinal cord injury. *J Transl Med* 10:174.

742 36. Hilt EE, McKinley K, Pearce MM, Rosenfeld AB, Zilliox MJ, Mueller ER, Brubaker  
743 L, Gai X, Wolfe AJ, Schreckenberger PC. 2014. Urine Is Not Sterile: Use of  
744 Enhanced Urine Culture Techniques To Detect Resident Bacterial Flora in the Adult  
745 Female Bladder. *Journal of Clinical Microbiology* 52:871-876.

746 37. Thomas-White KJ, Kliethermes S, Rickey L, Lukacz ES, Richter HE, Moalli P,  
747 Zimmern P, Norton P, Kusek JW, Wolfe AJ, Brubaker L. 2017. Evaluation of the  
748 urinary microbiota of women with uncomplicated stress urinary incontinence. *Am J*  
749 *Obstet Gynecol* 216:55.e1-55.e16.

750 38. Willner D, Low S, Steen JA, George N, Nimmo GR, Schembri MA, Hugenholtz P.  
751 2014. Single Clinical Isolates from Acute Uncomplicated Urinary Tract Infections  
752 Are Representative of Dominant In Situ Populations. *mBio* 5:e01064-13.

753 39. Siddiqui H, Nederbragt A, Lagesen K, Jeansson S, Jakobsen K. 2011. Assessing  
754 diversity of the female urine microbiota by high throughput sequencing of 16S rDNA  
755 amplicons. *BMC Microbiology* 11:244.

756 40. Nelson DE, Dong Q, Van Der Pol B, Toh E, Fan B, Katz BP, Mi D, Rong R,  
757 Weinstock GM, Sodergren E, Fortenberry JD. 2012. Bacterial Communities of the  
758 Coronal Sulcus and Distal Urethra of Adolescent Males. *PLoS ONE* 7:e36298.

- 759 41. Lewis DA, Brown R, Williams J, White P, Jacobson SK, Marchesi JR, Drake MJ.  
760 2013. The human urinary microbiome; bacterial DNA in voided urine of  
761 asymptomatic adults. *Frontiers in Cellular and Infection Microbiology* 3:41.
- 762 42. Price TK, Dune T, Hilt EE, Thomas-White KJ, Kliethermes S, Brincat C, Brubaker L,  
763 Wolfe AJ, Mueller ER, Schreckenberger PC. 2016. The Clinical Urine Culture:  
764 Enhanced Techniques Improve Detection of Clinically Relevant Microorganisms.  
765 *Journal of Clinical Microbiology* 54:1216-1222.
- 766 43. Khasriya R, Barcella W, De Iorio M, Swamy S, Gill K, Kupelian A, Malone-Lee J.  
767 2018. Lower urinary tract symptoms that predict microscopic pyuria. *International*  
768 *Urogynecology Journal* 29:1019-1028.
- 769 44. Alatoom AA, Cunningham SA, Ihde SM, Mandrekar J, Patel R. 2011. Comparison of  
770 direct colony method versus extraction method for identification of gram-positive  
771 cocci by use of Bruker Biotyper matrix-assisted laser desorption ionization-time of  
772 flight mass spectrometry. *J Clin Microbiol* 49:2868-73.
- 773 45. Griffiths RI, Whiteley AS, O'Donnell AG, Bailey MJ. 2000. Rapid Method for  
774 Coextraction of DNA and RNA from Natural Environments for Analysis of  
775 Ribosomal DNA- and rRNA-Based Microbial Community Composition. *Applied and*  
776 *Environmental Microbiology* 66:5488-5491.
- 777 46. Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK,  
778 Fierer N, Peña AG, Goodrich JK, Gordon JI, Huttley GA, Kelley ST, Knights D,  
779 Koenig JE, Ley RE, Lozupone CA, McDonald D, Muegge BD, Pirrung M, Reeder J,  
780 Sevinsky JR, Turnbaugh PJ, Walters WA, Widmann J, Yatsunenko T, Zaneveld J,  
781 Knight R. 2010. QIIME allows analysis of high-throughput community sequencing  
782 data. *Nature methods* 7:335-336.



- 783 47. DeSantis TZ, Hugenholtz P, Larsen N, Rojas M, Brodie EL, Keller K, Huber T,  
784 Dalevi D, Hu P, Andersen GL. 2006. Greengenes, a chimera-checked 16S rRNA gene  
785 database and workbench compatible with ARB. *Appl Environ Microbiol* 72:5069-72.
- 786 48. Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R. 2011. UCHIME improves  
787 sensitivity and speed of chimera detection. *Bioinformatics* 27:2194-2200.
- 788 49. Cole JR, Wang Q, Fish JA, Chai B, McGarrell DM, Sun Y, Brown CT, Porras-Alfaro  
789 A, Kuske CR, Tiedje JM. 2014. Ribosomal Database Project: data and tools for high  
790 throughput rRNA analysis. *Nucleic Acids Research* 42:D633-D642.
- 791 50. Mambatta AK, Jayarajan J, Rashme VL, Harini S, Menon S, Kuppusamy J. 2015.  
792 Reliability of dipstick assay in predicting urinary tract infection. *Journal of family  
793 medicine and primary care* 4:265-268.
- 794 51. Khasriya R, Khan S, Lunawat R, Bishara S, Bignall J, Malone-Lee M, Ishii H,  
795 O'Connor D, Kelsey M, Malone-Lee J. 2010. The inadequacy of urinary dipstick and  
796 microscopy as surrogate markers of urinary tract infection in urological outpatients  
797 with lower urinary tract symptoms without acute frequency and dysuria. *J Urol*  
798 183:1843-7.
- 799 52. Kupelian AS, Horsley H, Khasriya R, Amussah RT, Badiani R, Courtney AM,  
800 Chandhyoke NS, Riaz U, Savlani K, Moledina M, Montes S, O'Connor D, Visavadia  
801 R, Kelsey M, Rohn JL, Malone-Lee J. 2013. Discrediting microscopic pyuria and  
802 leucocyte esterase as diagnostic surrogates for infection in patients with lower urinary  
803 tract symptoms: results from a clinical and laboratory evaluation. *BJU Int* 112:231-8.
- 804 53. Brecher SM. 2016. Commentary: Complicated Urinary Tract Infections: What's a Lab  
805 To Do? *Journal of Clinical Microbiology* 54:1189-1190.
- 806 54. McNulty C. 2017. Public Health England: Management of infection guidance for  
807 primary care for consultation and local adaptation. United Kingdom.

- 808 [https://www.gov.uk/government/publications/managing-common-infections-](https://www.gov.uk/government/publications/managing-common-infections-guidance-for-primary-care)  
809 [guidance-for-primary-care.](https://www.gov.uk/government/publications/managing-common-infections-guidance-for-primary-care)
- 810 55. Bent S, Nallamotheu BK, Simel DL, Fihn SD, Saint S. 2002. Does this woman have an  
811 acute uncomplicated urinary tract infection? *Jama* 287:2701-10.
- 812 56. Swamy S, Barcella W, De Iorio M, Gill K, Khasriya R, Kupelian AS, Rohn JL,  
813 Malone-Lee J. 2018. Recalcitrant chronic bladder pain and recurrent cystitis but  
814 negative urinalysis: What should we do? *International Urogynecology Journal*  
815 29:1035-1043.
- 816 57. Dalal E, Medalia O, Harari O, Aronson M. 1994. Moderate stress protects female  
817 mice against bacterial infection of the bladder by eliciting uroepithelial shedding.  
818 *Infection and immunity* 62:5505-5510.
- 819 58. Rosen DA, Hooton TM, Stamm WE, Humphrey PA, Hultgren SJ. 2007. Detection of  
820 Intracellular Bacterial Communities in Human Urinary Tract Infection. *PLOS*  
821 *Medicine* 4:e329.
- 822 59. Smith YC, Rasmussen SB, Grande KK, Conran RM, O'Brien AD. 2008. Hemolysin  
823 of uropathogenic *Escherichia coli* evokes extensive shedding of the uroepithelium and  
824 hemorrhage in bladder tissue within the first 24 hours after intraurethral inoculation of  
825 mice. *Infect Immun* 76:2978-90.
- 826 60. Thumbikat P, Berry RE, Zhou G, Billips BK, Yaggie RE, Zaichuk T, Sun TT,  
827 Schaeffer AJ, Klumpp DJ. 2009. Bacteria-induced uroplakin signaling mediates  
828 bladder response to infection. *PLoS Pathog* 5:e1000415.
- 829 61. Hannan TJ, Mysorekar IU, Hung CS, Isaacson-Schmid ML, Hultgren SJ. 2010. Early  
830 severe inflammatory responses to uropathogenic *E. coli* predispose to chronic and  
831 recurrent urinary tract infection. *PLoS Pathog* 6:e1001042.

- 832 62. Chuang FC, Kuo HC. 2013. Increased urothelial cell apoptosis and chronic  
833 inflammation are associated with recurrent urinary tract infection in women. *PLoS*  
834 *One* 8:e63760.
- 835 63. Choi HW, Bowen SE, Miao Y, Chan CY, Miao EA, Abrink M, Moeser AJ, Abraham  
836 SN. 2016. Loss of Bladder Epithelium Induced by Cytolytic Mast Cell Granules.  
837 *Immunity* 45:1258-1269.
- 838 64. Gilbert NM, O'Brien VP, Lewis AL. 2017. Transient microbiota exposures activate  
839 dormant *Escherichia coli* infection in the bladder and drive severe outcomes of  
840 recurrent disease. *PLOS Pathogens* 13:e1006238.
- 841 65. Horsley H, Dharmasena D, Malone-Lee J, Rohn JL. 2018. A urine-dependent human  
842 urothelial organoid offers a potential alternative to rodent models of infection.  
843 *Scientific Reports* 8:1238.
- 844 66. Ragnarsdóttir B, Jönsson K, Urbano A, Grönberg-Hernandez J, Lutay N, Tammi M,  
845 Gustafsson M, Lundstedt A-C, Leijonhufvud I, Karpman D, Wullt B, Truedsson L,  
846 Jodal U, Andersson B, Svanborg C. 2010. Toll-Like Receptor 4 Promoter  
847 Polymorphisms: Common TLR4 Variants May Protect against Severe Urinary Tract  
848 Infection. *PLOS ONE* 5:e10734.
- 849 67. Brubaker L, Wolfe A. 2016. The urinary microbiota: a paradigm shift for bladder  
850 disorders? *Curr Opin Obstet Gynecol* 28:407-12.
- 851 68. Hornef M. 2015. Pathogens, Commensal Symbionts, and Pathobionts: Discovery and  
852 Functional Effects on the Host. *Ilar j* 56:159-62.
- 853 69. Bollgren I, Kallenius G, Nord CE, Winberg J. 1979. Periurethral anaerobic microflora  
854 of healthy girls. *J Clin Microbiol* 10:419-24.
- 855 70. Marrie TJ, Harding GK, Ronald AR. 1978. Anaerobic and aerobic urethral flora in  
856 healthy females. *J Clin Microbiol* 8:67-72.

- 857 71. Marrie TJ, Swantee CA, Hartlen M. 1980. Aerobic and anaerobic urethral flora of  
858 healthy females in various physiological age groups and of females with urinary tract  
859 infections. *J Clin Microbiol* 11:654-9.
- 860 72. Osset J, Bartolome RM, Garcia E, Andreu A. 2001. Assessment of the capacity of  
861 *Lactobacillus* to inhibit the growth of uropathogens and block their adhesion to  
862 vaginal epithelial cells. *J Infect Dis* 183:485-91.
- 863 73. Stapleton AE, Au-Yeung M, Hooton TM, Fredricks DN, Roberts PL, Czaja CA,  
864 Yarova-Yarovaya Y, Fiedler T, Cox M, Stamm WE. 2011. Randomized, Placebo-  
865 Controlled Phase 2 Trial of a *Lactobacillus crispatus* Probiotic Given Intravaginally  
866 for Prevention of Recurrent Urinary Tract Infection. *Clinical Infectious Diseases*  
867 52:1212-1217.
- 868 74. Le HT, Min B. 2016. Precision Targeting: Mast Cells Wipe Out Infected Bladder  
869 Epithelia. *Immunity* 45:1179-1181.
- 870 75. Xu H-S, Roberts N, Singleton FL, Attwell RW, Grimes DJ, Colwell RR. 1982.  
871 Survival and Viability of Nonculturable *Escherichia coli* and *Vibrio cholerae* in the  
872 Estuarine and Marine Environment. *Microbial Ecology* 8:313-323.
- 873 76. Nicolle LE. 2014. Catheter associated urinary tract infections. *Antimicrobial*  
874 *Resistance and Infection Control* 3:23.
- 875 77. Gottschick C, Deng Z-L, Vital M, Masur C, Abels C, Pieper DH, Wagner-Döbler I.  
876 2017. The urinary microbiota of men and women and its changes in women during  
877 bacterial vaginosis and antibiotic treatment. *Microbiome* 5:99.
- 878 78. Karstens L, Asquith M, Davin S, Stauffer P, Fair D, Gregory WT, Rosenbaum JT,  
879 McWeeney SK, Nardos R. 2016. Does the Urinary Microbiome Play a Role in  
880 Urgency Urinary Incontinence and Its Severity? *Front Cell Infect Microbiol* 6:78.

881 79. Chakravorty S, Helb D, Burday M, Connell N, Alland D. 2007. A detailed analysis of  
882 16S ribosomal RNA gene segments for the diagnosis of pathogenic bacteria. *Journal*  
883 *of microbiological methods* 69:330-339.

884 80. Jain M, Olsen HE, Paten B, Akeson M. 2016. The Oxford Nanopore MinION:  
885 delivery of nanopore sequencing to the genomics community. *Genome Biology*  
886 17:239.

887

888

889

890

891

892

893

894

895

896

897

898

899

900

901 **FIGURE LEGENDS**

902 **Figure 1.** Experimental workflow of the study. Abbreviations: MALDI-TOF MS = Matrix  
903 assisted laser desorption/ionisation time-of-flight mass spectrometry.

904 **Figure 2.** Composition types (no growth, pure and mixed cultures) achieved with three  
905 different techniques (MSU culture (1 µl), unspun culture (50 µl) and sediment culture (30 ml)  
906 across study groups.

907 **Figure 3.** Pairwise comparisons of the percentage frequencies of organisms identified from  
908 the urine specimens of new patients ( $n = 33$ ), relapsed patients ( $n = 30$ ) and controls ( $n = 29$ )  
909 using three different culture techniques: routine midstream urine (MSU) culture, unspun  
910 culture of a 50 µl sample volume and a spun sediment culture of a 30 ml sample volume. **3A:**  
911 new patients versus controls, **3B:** relapsed patients versus asymptomatic controls and **3C:**  
912 new patients versus controls. Data are presented as percentages of the total number of isolates  
913 identified.

914 **Figure 4.** Percentage sequence abundances of the 20 most abundant taxa detected in both  
915 new patient and control cohorts when categorised by sample type.

916 **Figure 5.** Comparison of genus-level taxa detected by sediment culture and 16S rRNA gene  
917 sequencing for new patient (NL) spun samples ( $n = 33$ ) and asymptomatic control (AC) spun  
918 samples ( $n = 29$ ). Interpretation: bacteria identified by culture only (pink), sequencing only  
919 (blue), both (purple) and neither (cream).

920

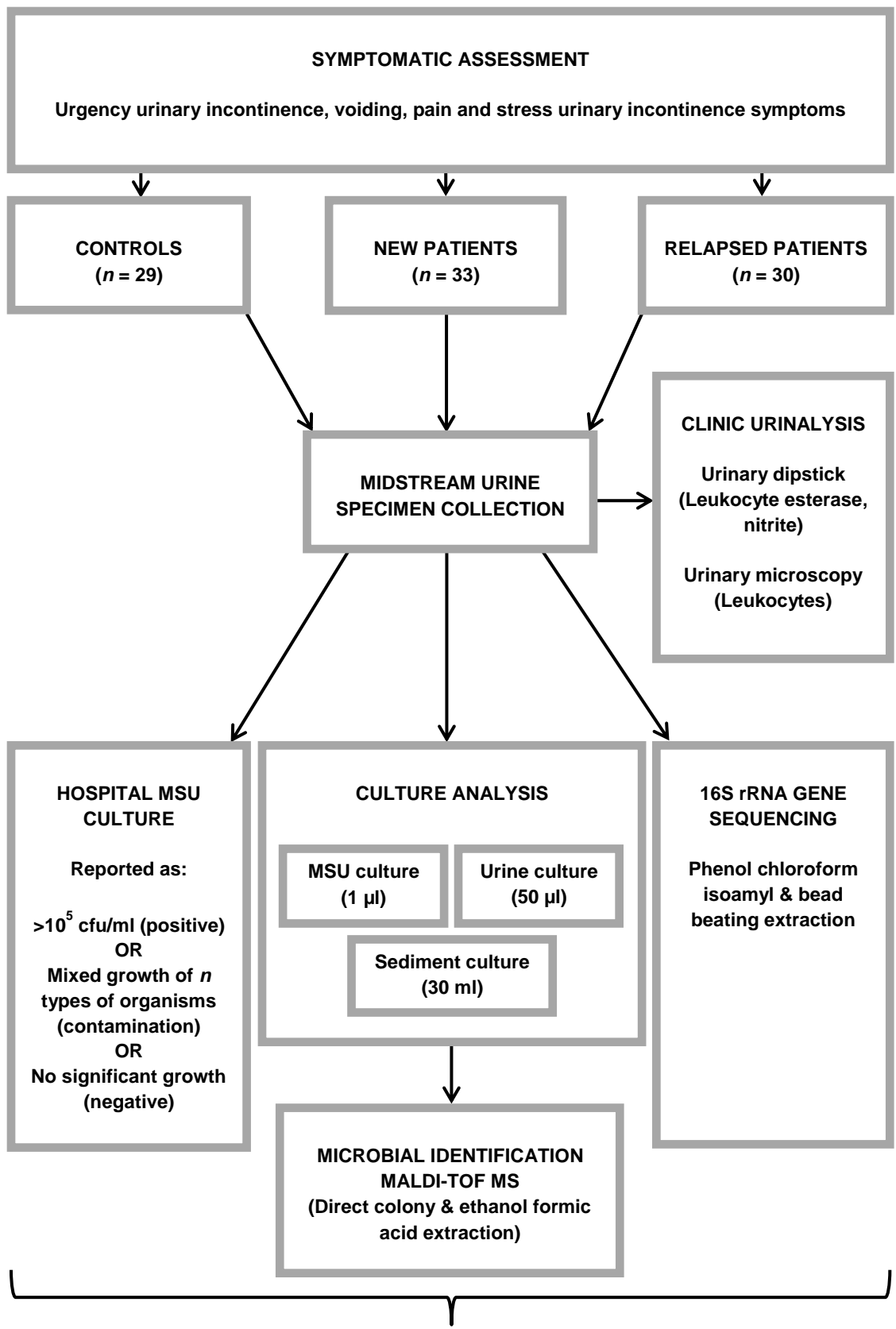
921

922

923

924  
925  
926  
927  
928  
929  
930  
931  
932  
933  
934  
935  
936  
937  
938  
939  
940  
941  
942

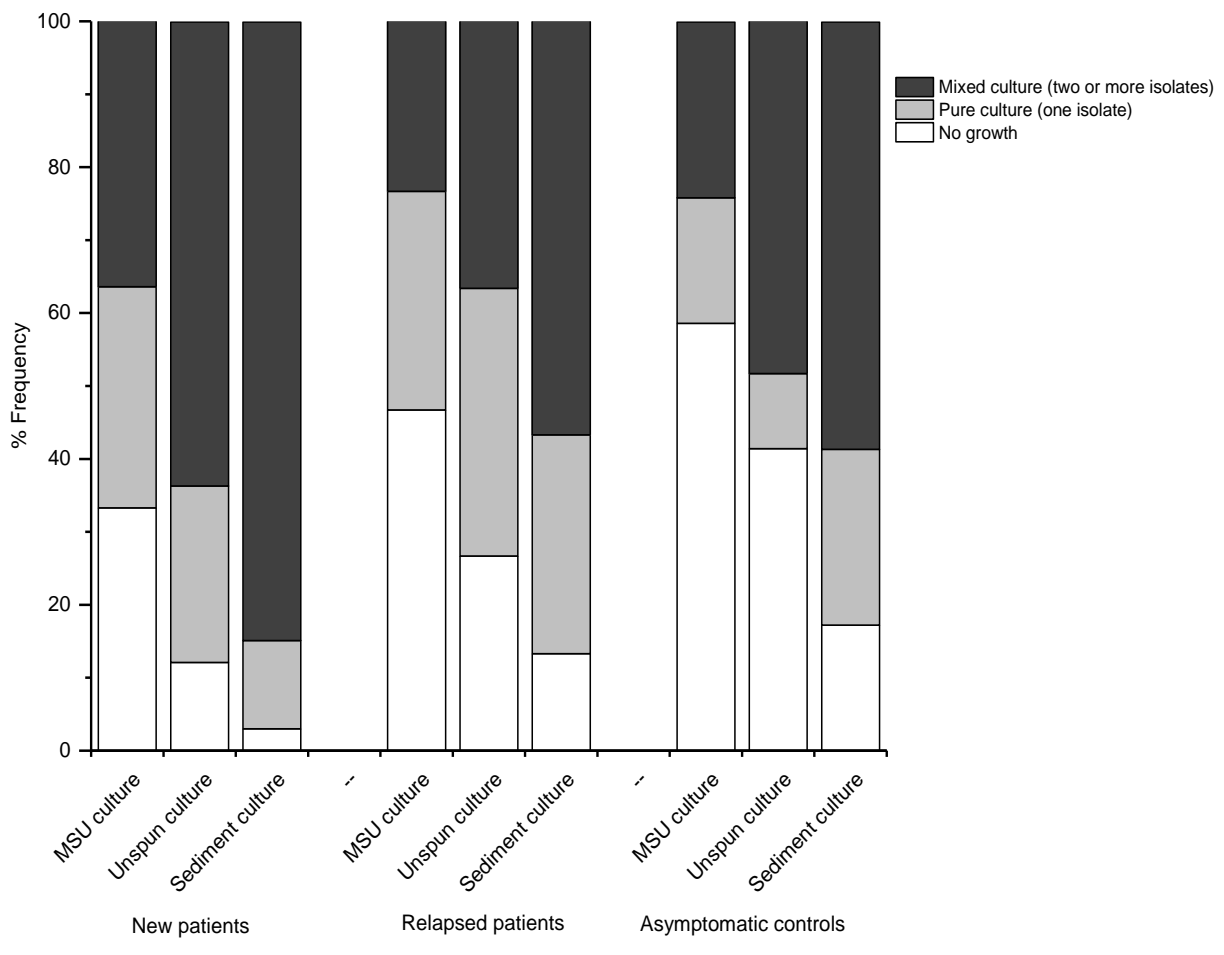
OBJECTIVE 1: Evaluation of UK MSU culture across symptomatic and asymptomatic groups



OBJECTIVE 2: Comparison of MSU culture with alternative methods for bacterial enrichment

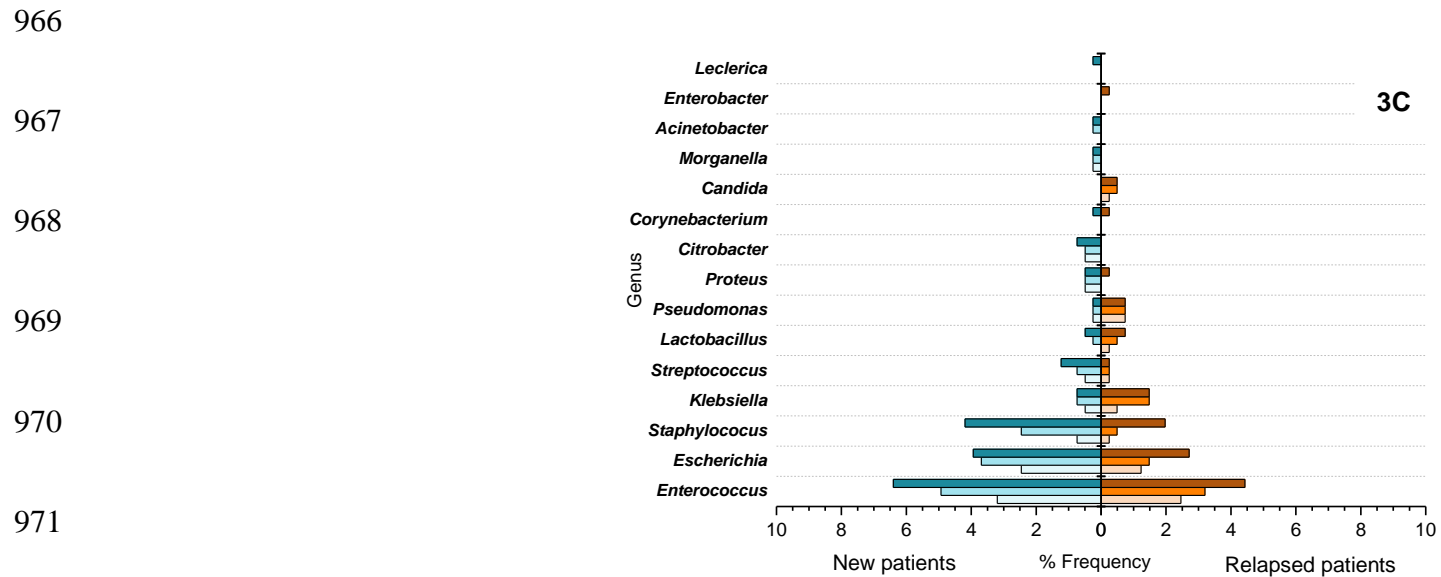
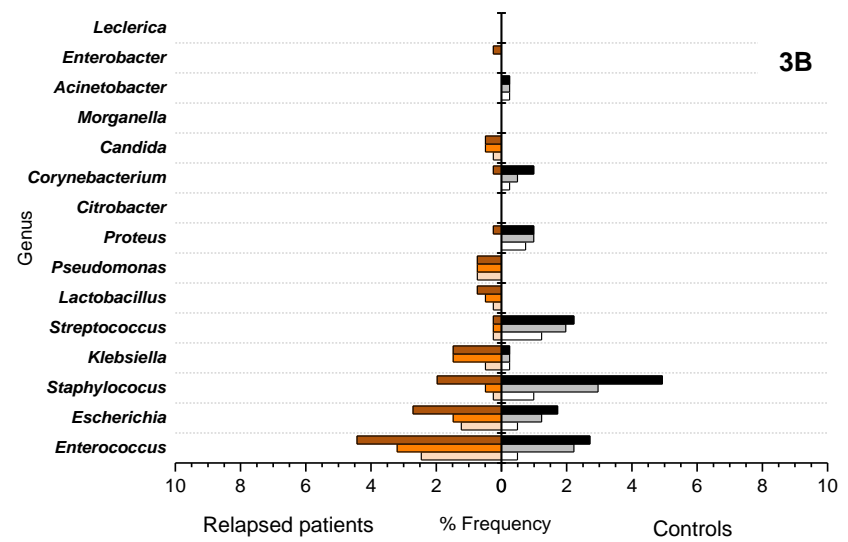
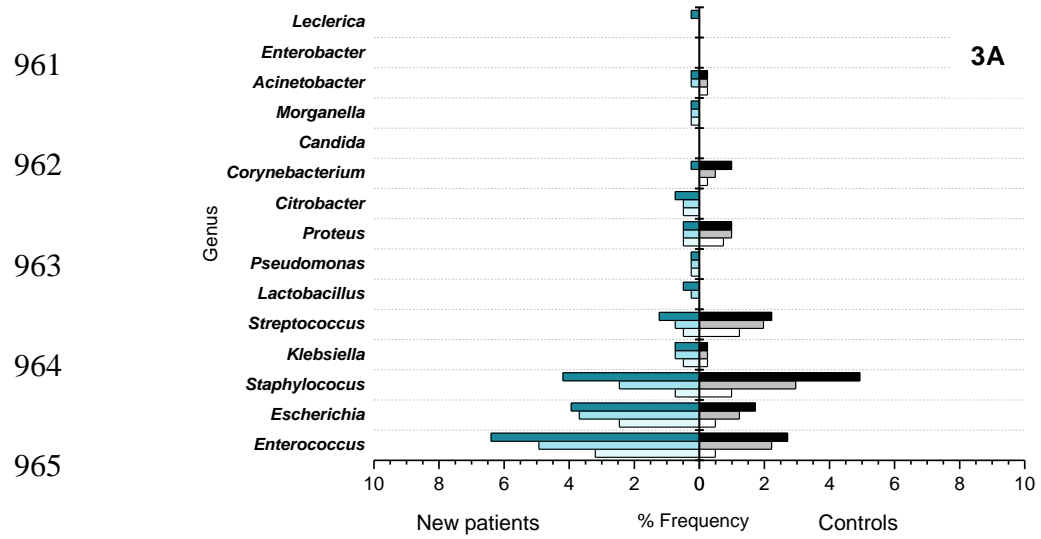
Figure 1.

943  
944  
945  
946  
947  
948  
949  
950  
951  
952  
953  
954  
955  
956  
957  
958  
959  
960

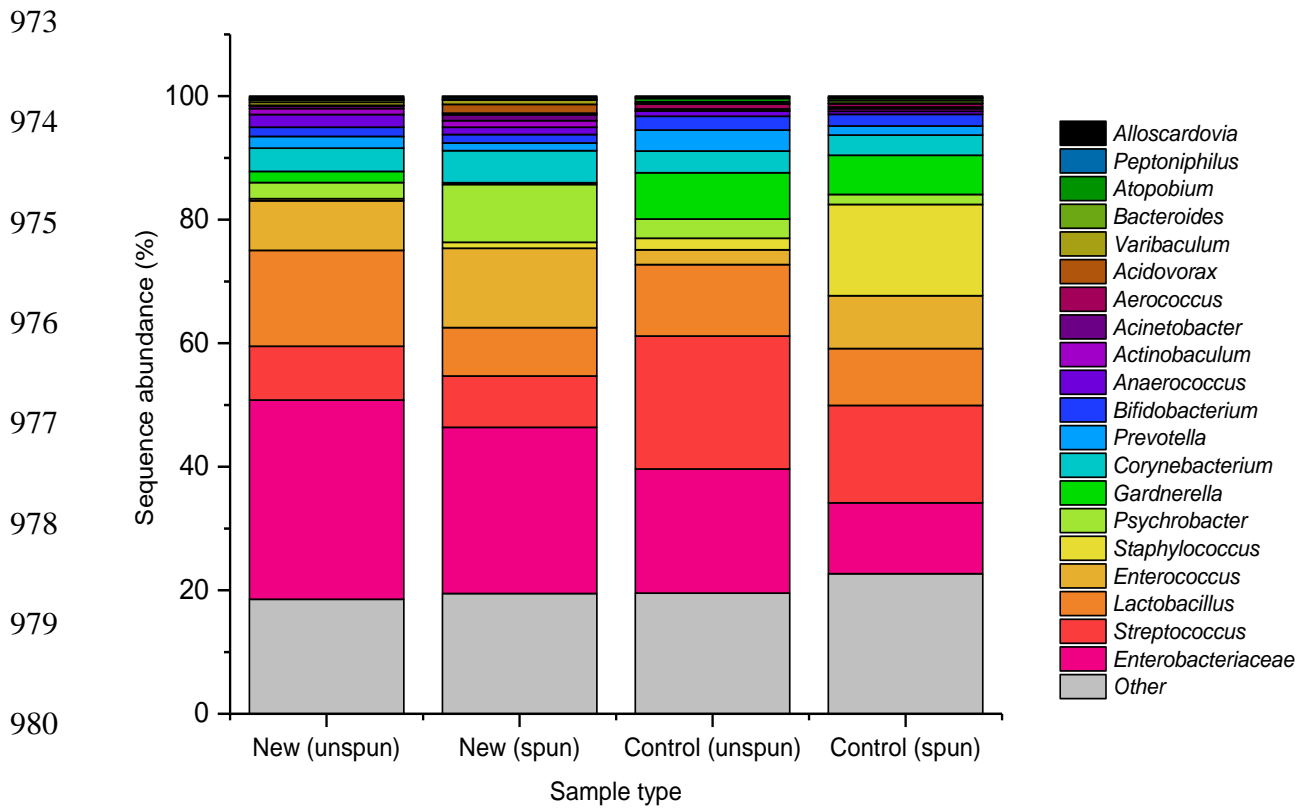


**Figure 2.**



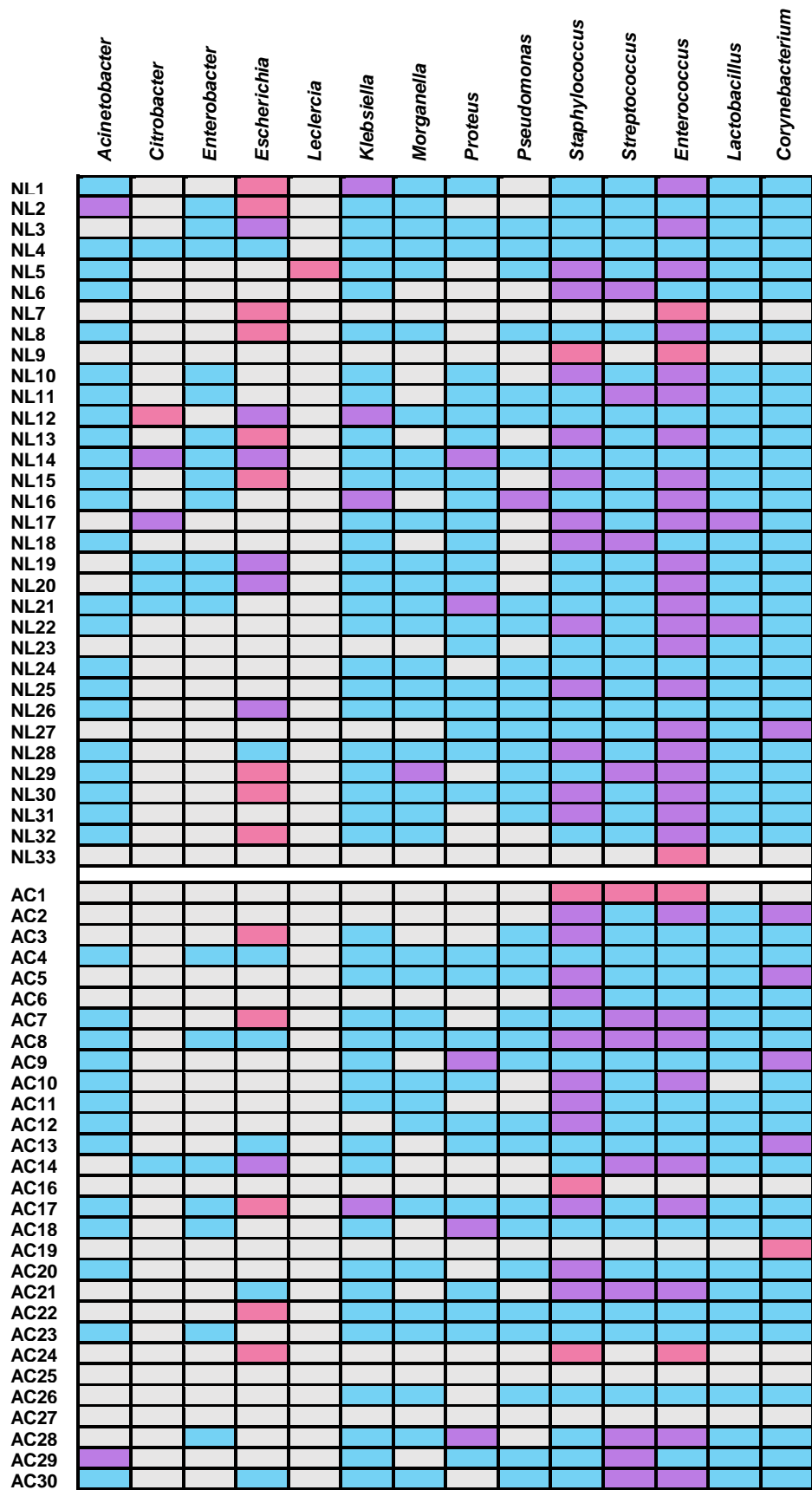


972 **Figure 3.**



991 **Figure 4.**

992  
993  
994  
995  
996  
997  
998  
999  
1000  
1001  
1002  
1003  
1004  
1005  
1006  
1007  
1008  
1009



1010 **Figure 5.**

1011 **TABLES**

1012 **Table 1.** Clinical characteristics of each study group. Abbreviations: CI = confidence  
1013 interval, SD = standard deviation, SUI = stress urinary incontinence, UUI = urgency urinary  
1014 incontinence. Superscripts: a = Kruskal-Wallis test, b = Mood's median test, c = Chi-squared  
1015 test. \$ = statistical comparison between new patient and relapsed patient groups only.

1016 **Table 2.** Descriptive measures of in-house (clinic) microscopic leukocyte counts and reported  
1017 routine hospital midstream urine culture results for each study group. Abbreviations: wbc =  
1018 white blood cell.

1019 **Table 3.** Richness and diversity measures determined for new patients and controls

1020

1021

1022

1023

1024

1025

1026

1027

1028

1029

1030

1031 **Table 1.**

<b>CHARACTERISTIC</b>	<b>NEW PATIENTS (n = 33)</b>			<b>RELAPSED PATIENTS (n = 30)</b>			<b>CONTROLS (n = 29)</b>			<b>P-VALUE</b>
<b>Demographics</b>										
Female (%)	32 (97.0)			27 (90.0)			26 (89.7)			
Male (%)	1 (3.0)			3 (10.0)			3 (10.3)			
Mean age in years (SD)	48.7 (16.5)			47.8 (16.5)			40.7 (15.7)			0.113 <sup>a</sup>
Age range	18-77			24-78			20-76			
<b>Urinary Patterns (24 hours)</b>	<b>Mean</b>	<b>SD</b>	<b>Median (95% CI)</b>	<b>Mean</b>	<b>SD</b>	<b>Median (95% CI)</b>	<b>Mean</b>	<b>SD</b>	<b>Median (95% CI)</b>	
Frequency	8.8	5.1	6.5 (5.5-10.5)	8.5	3.7	8.0 (6.5-9.5)	5.9	1.5	5.5 (5.5-6.5)	< 0.05 <sup>b</sup>
Nocturia	1.7	1.7	1.5 (0.5-2.0)	1.6	1.9	1.0 (0.0-2.5)	0.3	0.4	0.0 (0.0-0.5)	< 0.05 <sup>b</sup>
<b>Symptoms<sup>§</sup> [Yes/No] (% within group)</b>										
UUI (%)	25 (75.8)			12 (40.0)			0 (0.0)			< 0.05 <sup>c</sup>
Pain (%)	24 (72.7)			26 (86.7)			0 (0.0)			0.172 <sup>c</sup>
Voiding (%)	29 (87.9)			21 (70.0)			0 (0.0)			0.080 <sup>c</sup>
SUI (%)	17 (51.5)			2 (6.7)			0 (0.0)			< 0.001 <sup>c</sup>
<b>Number of symptoms</b>	<b>Mean</b>	<b>SD</b>	<b>Median (95% CI)</b>	<b>Mean</b>	<b>SD</b>	<b>Median (95% CI)</b>	<b>Mean</b>	<b>SD</b>	<b>Median (95% CI)</b>	
UUI symptoms	3.4	2.9	3.0 (1.0-4.0)	1.4	2.2	0.0 (0.0-2.0)	0.0	0.0	0.0 (0.0-0.0)	< 0.001 <sup>b</sup>
Pain symptoms	3.1	2.7	3.0 (1.0-4.0)	3.6	2.9	3.0 (2.0-4.0)	0.0	0.0	0.0 (0.0-0.0)	< 0.001 <sup>b</sup>
Voiding symptoms	4.1	2.8	4.0 (2.0-6.0)	3.6	2.8	4.0 (2.0-5.0)	0.0	0.0	0.0 (0.0-0.0)	< 0.001 <sup>b</sup>
SUI symptoms	0.7	0.8	1.0 (0.0-1.0)	0.3	1.0	0.0 (0.0-0.0)	0.0	0.0	0.0 (0.0-0.0)	< 0.001 <sup>b</sup>
Total symptom score	11.3	5.9	12.0 (9.0-14.0)	8.9	5.2	9.0 (6.0-11.0)	0.0	0.0	0.0 (0.0-0.0)	< 0.001 <sup>b</sup>

1032

1033

1034

1035 **Table 2.**

1036

1037

<b>DIAGNOSTIC TEST</b>	<b>NEW PATIENTS (n = 33)</b>	<b>RELAPSED PATIENTS (n = 30)</b>	<b>CONTROLS (n = 29)</b>
<b>CLINIC MICROSCOPIC LEUKOCYTES (log<sub>10</sub>wbc/μl)</b>			
Mean log <sub>10</sub> cell count, SD (95% CI)	1.7, 0.9 (0.3-1.0)	0.7, 1.0 (0.4-1.1)	0.2, 0.4 (0.1-0.3)
Median log <sub>10</sub> cell count (95% CI)	0.3 (0.0-0.3)	0.5 (0.0-0.8)	0.0 (0.0-0.8)
<b>Frequencies (% of group)</b>			
zero wbc/μl	15 (45.5)	12 (40.0)	20 (69.0)
1-9 wbc/μl	11 (33.3)	11 (36.7)	8 (27.6)
≥10 wbc/μl	7 (21.2)	7 (23.3)	1 (3.4)
<b>HOSPITAL MIDSTREAM URINE CULTURE</b>			
No significant growth <sup>\$</sup>	21 (63.6)	23 (76.7)	22 (75.9)
Mixed growth <sup>*</sup> of two organisms	3 (9.1)	2 (6.7)	4 (13.8)
Mixed growth of three organisms	4 (12.1)	2 (6.7)	1 (3.4)
>10 <sup>5</sup> cfu/ml of one organism	4 (12.1)	2 (6.7)	2 (6.9)

1045

1046 Footnotes:

1047 \$ = <10<sup>5</sup> organisms/ml, **or** growth of ≥2 organisms at <100 colonies

1048 \* = >100 colonies

1049

1050

1051 Table 3.

<b>METRIC</b>	<b>NEW PATIENTS</b>			<b>CONTROLS</b>		
	<b>Unspun (n = 30)</b>	<b>Spun (n = 30)</b>	<b>Both (n = 60)</b>	<b>Unspun (n = 22)</b>	<b>Spun (n = 23)</b>	<b>Both (n = 45)</b>
<b>OTU Numbers</b>						
Mean (SD)	237.5 (122.4)	243.5 (145.1)	247.0 (137.7)	271.8 (271.8)	264.7 (235.2)	276.2 (214.0)
Median	251.0	224.0	247.5	247.0	217.5	230
Min-Max	4-510	7-606	4-619	10-639	6-1016	6-1016
<b>Chao1 estimator</b>						
Mean (SD)	310.2 (147.7)	317.0 (159.7)	333.3 (164.4)	341.6 (205.0)	336.9 (253.0)	362.5 (255.9)
Median	340.9	283.4	334.2	315.1	295.6	321.1
Min-Max	7-570.5	7.2-706.0	7-740.6	20.5-780.2	7.5-1085.7	7.5-1193.4
<b>Shannon's index</b>						
Mean (SD)	1.6 (0.8)	1.7 (0.9)	1.7 (0.9)	1.8 (0.8)	1.7 (0.8)	1.7 (0.8)
Median	1.5	1.8	1.7	1.9	1.7	1.8
Min-Max	0.3-3.3	0.5-3.3	0.3-3.3	0.4-3.2	0.3-3.4	0.3-3.4
<b>Inverse Simpson's Index</b>						
Mean (SD)	3.4 (2.8)	4.0 (3.2)	3.7 (3.0)	3.9 (2.6)	3.9 (3.5)	3.9 (3.0)
Median	2.6	3.0	2.7	3.3	2.5	3.1
Min-Max	1.1-14.6	1.2-13.2	1.1-14.6	1.1-11.6	1.1-17.0	1.1-17.0