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1	Co-culture of Methanobrevibacter smithii with enterobacteria during urinary infection.
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24 Summary

Background. Urinary tract infections are known to be caused by bacteria, but the potential
implications of archaea have never been studied in this context.

27 Methods. In two different university hospital centres we used specific laboratory methods for

the detection and culture of archaeal methanogens in 383 urine specimens prospectively

29 collected for diagnosing urinary tract infection (UTI).

30 Findings. *Methanobrevibacter smithii* was detected by quantitative PCR and sequencing in

31 34 (9%) of the specimens collected from 34 patients. *Escherichia coli, Klebsiella*

32 *pneumoniae*, *Enterobacter* sp., *Enterococcus faecium* and mixed cultures were detected along

33 with *M. smithii* in eighteen, six, three, one and six urine samples, respectively. Interestingly,

using our specific culture method for methanogens, we also isolated *M. smithii* in 31 (91%) of

the 34 PCR positive urine samples. Genotyping the 31 isolates using multispacer sequence

typing revealed three different genotypes which have been previously reported in intestinal

37 microbiota. Antibiotic susceptibility testing found the 31 isolates to be *in vitro* susceptible to

38 metronidazole (MIC: 1 mg/L) but resistant to fosfomycin, sulfamethoxazole-trimethoprim,

amoxicillin-clavulanate and ofloxacin, commonly used to treat bacterial UTI. Finally, 19

40 (54%) of the 34 patients in whose urine samples *M. smithii* was detected were diagnosed with

41 UTIs, including cystitis, pyelonephritis and prostatitis.

Interpretation. Our results show that *M. smithii* is part of the urinary microbiota of some
individuals and could play a role in community-acquired UTI in association with enteric
bacteria.

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49 Introduction

Forty-eight-hour culturing of a large volume of urine samples collected from women by
transurethral catheter in parallel with suprapubic aspiration has shown that urine hosts a
resident microbiota, which includes bacteria missed by routine procedures¹. This resident
urinary microbiota has been further explored using an expanded quantitative urine culture
(EQUC) method^{2,3}. Metagenomic analyses have confirmed that urine is not sterile in healthy
individuals⁴⁻⁸.

An interesting concept currently emerging is that cases of urinary tract infection (UTI) may result from disequilibrium in the repertoire of the urinary microbiota rather than from the introduction of an exogenous pathogenic organism^{8,9}. Therefore, efforts to characterise the repertoire of organisms residing in the urinary microbiota are important for clinical microbiology and medicine.

61 Current culture-dependant and culture-independent studies of the urinary microbiota 62 rely on methods that are unable to detect archaea. These micro-organisms form a living 63 domain distinct from that of bacteria and have been detected and cultured from the oral and 64 gut microbiota¹⁰⁻¹². Moreover, the specific archaea group of methanogens, characterised by 65 the ability to produce methane from hydrogen, are recognised as emerging pathogens in a few 66 clinical situations, including life-threatening brain abscess ^{12-15.}

In this study, we aimed to characterise the repertoire of human urine microbiota using laboratory protocols specifically designed for the detection and culture of methanogens in urines. Surprisingly, we found that among the six previously-described methanogens in nonurine human microbiota, only *Methanobrevibacter smithii* can be detected and cultured from urines.

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74 **Patients and methods**

75 Sample collection. The retrospective study was approved by the Ethics Committee of the IHU Méditerranée Infection under n° 2016-01. A total of 383 urine specimens taken for 76 77 microbiological diagnosis of UTI in 383 patients without urinary catheter were prospectively collected at the IHU Méditerranée Infection Laboratory, Marseille, France (henceforth 78 referred to as laboratory 1) (183 urines) and at the Laboratory of Bacteriology at the 79 80 University Centre of Nice (henceforth referred to as laboratory 2) (200 urines) using a BD Vacutainer (Becton Dickinson, Le Pont de Claix, France). In this study, clean catch 81 midstream urine specimen were collected by from September 2017 to November 2017 at 82 83 laboratory 1 and from January 2018 to March 2018 at laboratory 2. A total of 50 BD vacutainer tubes filled with sterile phosphate buffered saline (PBS) (Thermo Fisher Scientific, 84 Villebon-sur-Yvette, France) were intercalated in every 8 urine specimens to serve as negative 85 controls in all further laboratory steps. The following data on each patient included in the 86 study were anonymously collected: age, sex, underlying disease of the urinary tract, immune 87 88 status, symptoms of urinary tract infection, antibiotic treatment (molecule and duration) and clinical outcome. 89

Routine investigations. Urine leukocyte and erythrocyte counts were obtained with a UF-90 1000i[®] analyser (Sysmex, bioMérieux, Craponne, France) in laboratory 1 and with an IQ 200 91 IRIS analyser (Beckman Coulter, Villepinte, France) in laboratory 2. Gram staining was 92 carried out with the automated PREVI Color Gram (bioMérieux) at both laboratories in order 93 to stain bacteria. Indeed, methanogens are not usually coloured by using Gram staining. A 94 200-uL urine volume was cultured on a COLUMBIA COS medium (bioMérieux) (laboratory 95 96 1) or on Uriselect medium (Bio-Rad, Marnes-la-Coquette, France) (laboratory 2) and incubated at 37 °C for 24 h (both laboratories), while visible colonies were identified by 97

- 98 matrix-assisted laser desorption ionisation-time of flight mass spectrometry (Brucker,
- 99 Wissembourg, France), as previously described¹⁶.

Methanogen DNA extraction, PCR and sequencing. The manual protocol was carried out
 using the NucleoSpin® Tissue Mini Kit (Macherey Nagel, Hoerdt, France) as previously
 described^{11,17}. Extraction of 250 µL of sterile PBS was included in each DNA extraction
 series as a negative control.

104 Methanogen PCR and sequencing was performed as described in previous articles^{11,17} using

105 the 16S rRNA broad range archaeal forward primer 5'-CCGGGTATCTAATCCGGTTC- 3'

and reverse primer 5'-CTCCCAGGGTAGAGGTGAAA-3¹⁸, and the *mcr*A gene forward

107 primer 5'-GCTCTACGACCAGATMTGGCTTGG-3' and reverse primer 5'-

108 CCGTAGTACGTGAAGTCATCCAGCA-3^{,19}. All PCR products were sequenced and the

109 various fragments were assembled using the ChromasPro1.34 software (Technelysium Pty.

110 Ltd., Tewantin, Australia) and compared with sequences available in the GenBank database

- using the online NCBI BLAST program (http://blast.ncbi.nlm.nih.gov.gate1.inist.fr/Blast.cgi).
- 112 Extracted DNA was also analysed by real-time PCR targeting *M. smithii* using the following
- 113 primer pair: M. smithii-F (5'-ACCATAACyATCAGCAGCATTAT-3') and M. smithii-R (5'-

114 AGTATTGGTGAAGGATTTaCTGT-3') (Eurogentec, Seraing, Belgium) and the *M. smithii*

115 probe (6-carboxyfluorescein [FAM]-5'ACCyTTATCAGCTTTACCA TTAATyAAAG-3')

116 (Applied Biosystems, Courtaboeuf, France) as described in a previous study¹³.

Methanogen isolation and culture. A 250 µL volume of urine sample was seeded in ambient
air in a sterile Hungate tube²⁰ (Dominique Dutscher, Brumath, France). The Hungate culture
tube was developed specifically for growing and storing strictly anaerobic bacteria and
archaea. It features an autoclavable screw cap with a 9-mm opening, a non-toxic, gasimpermeable butyl rubber stopper and a disposable screw cap. We placed 5 mL of SAB

broth²¹ into each Hungate tube, seeded it with urine then inoculated it with *Bacteroides*

thetaiotaomicron (10^5 cells/mL) for hydrogen production²². The mixture in the Hungate tube 123 was then incubated at 37 °C with agitation for seven days. Methanogen growth was inferred 124 from the production of CH_4 detected by gas chromatography, as previously described¹¹. The 125 subculture was seeded on a Petri dish containing SAB medium supplemented with 15 g/L 126 agar and placed in the upper chamber of a double-chamber box. Tubes inoculated with sterile 127 PBS were used as negative controls in the archaea isolation experiments (one negative control 128 every five tubes); culture dishes containing the SAB medium inoculated with sterile PBS were 129 used as negative controls in the sub-culture experiments (one negative control every five 130 dishes). 131

Characterisation of isolates. Isolates were further genotyped using the Multi Spacer Typing
method, as previously described²³. In addition, we determined the antibiotic susceptibility
profile of the isolates, as previously described, by incorporating the following antibiotics:
fosfomycin (100 mg/L), sulfamethoxazole-trimethoprim (50 mg/L), amoxicillin-clavulanate
(100 mg/L) and ofloxacin (1 mg/L) (BIOGARAN, Colombes, France)²¹. *M. smithii* culture
with metronidazole (1 mg/L) was used as the positive control and *M. smithii* culture without
metronidazole as the negative control.

Statistical analyses. Data were analysed with Prism 7.0 (GraphPad Software) by unpaired Student's t-test and chi-square test (** p < 0.01, * p < 0.05, ns: non-significant). We used the former to compare the quantitative variables from the two centres (pH, urine salinity, white and red blood cells), and the latter to compare the categorical variables (sex ratio, % of standard cultures positive, % of urine samples positive for methanogens by molecular or culture methods).

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148 **Results**

Population and routine investigations. A total of 383 urine specimens from 383 patients
were collected and analysed prospectively, 183 specimens in laboratory 1 and 200 specimens
in laboratory 2. Mean patient age was 56 years (0-95 years) and 61% of the patients were
women. Leukocyturia was significant in 205 of the 383 specimens (> 10⁴ leukocytes/mL) and
142 specimens yielded bacterial growth. Comparison of the data collected from laboratory 1
and from laboratory 2 is shown in Table 1.

Methanogen quantitative PCR and sequencing. Of the 383 urine samples analysed by 155 standard PCR sequencing, 18/183 (10%) from laboratory 1 and 16/200 (8%) from laboratory 156 157 2 were positive for both archaeal 16S rRNA PCR and the mcrA PCR, while 165/183 (90%) and 184/200 (92%), respectively, were negative in both PCR assays in the presence of 50 158 159 negative controls, all of which remained negative. Sequencing the 16S rRNA PCR products revealed 99% sequence similarity with the homologous fragment of the reference 16S rRNA 160 161 gene of M. smithii strain NVD (accession NCBI: LT223565). Further sequencing of the PCR-162 amplified mcrA gene revealed 99% sequence similarity with the homologous fragment of the reference mcrA gene of M. smithii strain NVD (accession NCBI: LT223565) and M. smithii 163 ATCC 35061 (accession NCBI: NR_074235). Quantitative PCR analyses targeting the M. 164 165 smithii 16S rRNA gene yielded a median Ct of 33.7 ± 2.47 , indicative of an M. smithii load of $1.24 \times 10^3 \pm 1.56 \times 10^3$ / mL. Detection of *M. smithii* in urine samples was significantly 166 associated with leukocyte counts greater than 10^4 /mL, P-value = 0.0015 (Chi-square test). 167 Isolation and culture of *M. smithii*. To test the viability of the *M. smithii* organisms detected 168 by the PCR-based methods, methanogen was cultured on 18 samples in laboratory 1 and 16 169 170 samples in laboratory 2. Fifteen of the 18 cultured samples in laboratory 1 and all 16 cultured samples in laboratory 2 yielded colonies, which were obtained after 20 days of incubation. All 171 the colonies were identified as M. smithii on the basis of PCR sequencing of the 16S rRNA 172

and *mcr*A genes, which revealed 100% sequence similarity with the reference homologous
genes in *M. smithii* strain NVD (accession NCBI: LT223565) (Table 1). MST genotyping
indicated that the *M. smithii* isolates belonged to genotype 2 (n=13), genotype 3 (n=10) and
genotype 1 (n=8) (Supplementary Table 1). Antibiotic susceptibility testing indicated that
fosfomycin (100 mg/L), sulfamethoxazole-trimethoprim (50 mg/L), amoxicillin-clavulanate
(100 mg/L) and ofloxacin (1 mg/L) were inactive against *M. smithii*, whereas metronidazole used as a positive control - inhibited the growth of *M. smithii*.

Microbiological and clinical analyses. *M. smithii* was detected in 34 (9%) of the 383 urine

181 samples analysed in the study. *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter* sp. and

182 Enterococcus faecium were detected along with M. smithii in 18, 6, 3 and 1 urine samples,

respectively. The other six samples in which *M. smithii* was detected yielded mixed cultures

including *Enterobacteriaceae*. Of the 34 patients with *M. smithii* in their urine samples,

185 63.4% were women, and mean patient age was 63 years (20-95). Ten patients (30%) had an

underlying urinary tract disease, 14 (40%) were immunosuppressed and 19 (44%) had chronic

renal failure (GFR < 60L/min). It is worth noting that *M. smithii* was associated with UTI in

188 19 (56%) patients and with colonisation in 15 (44%). Of the 19 patients with UTIs, 17 (88%)

189 were community acquired, 13 presented with cystitis, while four were diagnosed with

190 pyelonephritis and two with prostatitis. All 19 patients received antibiotic treatments targeting

191 the isolated bacteria and commonly used to treat UTIs, including beta-lactams,

192 fluoroquinolones and fosfomycin-trometamol, with favourable outcomes (Table 2).

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198 **Discussion**

M. smithii was the only methanogen prospectively detected in the urines using a 199 polyphasic approach, including molecular detection by PCR sequencing of two unrelated 200 genes, and isolation and culture methods specifically developed for methanogenic archaea. 201 Given that the negative controls included in every experimental procedure remained negative, 202 that *M. smithii* was detected by different unrelated technical approaches, and that similar data 203 were obtained in two unrelated laboratories, we can exclude the possibility of in-laboratory 204 205 contamination and centre bias in the study. These pieces of experimental evidence, therefore, validate the data reported here. 206

207 *M. smithii* was first detected as a normal organism in the gut microbiota of the vast majority of individuals^{11,24}, although it is depleted in children diagnosed with severe 208 malnutrition and kwashiorkor 25 . It was then shown to be a component of the oral cavity 209 microbiota in the saliva¹⁷ and the dental plaque, including periodontitis^{26,27} and peri-210 implantitis lesions^{10,28}. More recently, the pathogenic potential of M. smithii was illustrated by 211 its isolation and culture from a muscular abscess¹⁴ and by its detection in one case of brain 212 abscess¹³. Our study is of interest because it reports for the first time *M. smithii*, methanogens 213 and archaea at large in urines, as all previous culture-based and culture-independent studies 214 have failed to detect archaea in this type of fluid. Our success in detecting M. smithii was 215 216 down to the use of laboratory tools that we developed specifically for detecting methanogens^{22, 29-31}. These tools, including the technique we set up for easy isolation and 217 culture of methanogens, could be easily implemented in other clinical microbiology 218 laboratories enabling them to investigate the presence of methanogens in urines 30 . 219 We detected *M. smithii* in 9% of urine samples, and, furthermore, in the same 220 proportions of patients in two unrelated laboratories (10% in laboratory1 and 8% in laboratory 221 2). This supports the idea that *M. smithii* may be part of "the urinary microbiota", a new 222

concept that has emerged since the expansion of urine metagenomic analyses and EQUC 223 224 approaches. It is worth noting that all the urine samples which cultured *M. smithii* also cultured enterobacteria, such as E. coli, K. pneumoniae, Enterobacter sp., which are known to 225 produce hydrogen as a substrate for methanogenesis³². The fact that *M. smithii* was isolated 226 along with enterobacteria and never detected in a urine sample without bacterial growth 227 suggests that it could play a role in or even induce dysbiosis, which facilitates the growth of 228 229 enterobacteria, acknowledged agents of UTI. In our study, 19 (54%) of the 34 patients with *M. smithii* in their urine samples presented with UTIs, were most of them community 230 acquired. These preliminary results need to be confirmed by further studies, including 231 epidemiological, clinical and fundamental approaches, in order to determine the role of M. 232 smithii in UTIs. Finally, our study indicates that the antibiotics commonly used to treat 233 bacterial urinary tract infections are inactive against *M. smithii*, consistent with a previous 234 report of its narrow spectrum of *in vitro* susceptibility³³. Interestingly, the 19 patients in 235 whose urine samples we detected *M. smithii* and who presented with UTI symptoms showed 236 237 clinical improvement after receiving antimicrobial treatment active against enterobacteria but 238 inactive against methanogens. We hypothesise that the inhibition of enterobacterial growth by appropriate antibiotic treatment induces a decrease ²in hydrogen production, a major substrate 239 240 for methanogenesis, and therefore destroys the metabolic cooperation between these two microorganisms. 241

In conclusion, our data show that *M. smithii* is part of the urinary microbiota in some patients and suggest that it plays a role in community-acquired UTIs in association with enterobacteria. Therefore, we encourage colleagues to use the above-described methods to investigate methanogens in urines, including *M. smithii*, in order to determine its potential contribution to the pathogenesis of UTIs.

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References

249	1- Wolfe AJ, Toh E, Shibata N, Rong R, Kenton K, FitzGerald MP, et al. Evidence of
250	uncultivated bacteria in the adult female bladder. <i>J Clin Microbiol</i> 2012; 50 :1376–83.
251	2-Hilt EE, McKinley K, Pearce MM, Rosenfeld AB, Zilliox MJ, Mueller ER, et al. Urine is
252	not sterile: Use of enhanced urine culture techniques to detect resident bacterial flora in
253	the adult female bladder. <i>J Clin Microbiol</i> 2014; 52 :871–6.
254	3-Xu W, Yang L, Lee P, Huang WC, Nossa C, Ma Y, et al. Mini-review: perspective of the
255	microbiome in the pathogenesis of urothelial carcinoma. Am J Clin Exp Urol
256	2014; 2 :57–61.
257	4-Siddiqui H, Nederbragt AJ, Lagesen K, Jeansson SL, Jakobsen KS. Assessing diversity of
258	the female urine microbiota by high throughput sequencing of 16S rDNA amplicons.
259	<i>BMC Microbiol</i> 2011; 11 :244.
260	5-Schneeweiss J, Koch M, Umek W. The human urinary microbiome and how it relates to
261	urogynecology. Int Urogynecol J 2016;27:1307–12.
262	6-Bao Y, Al KF, Chanyi RM, Whiteside S, Dewar M, Razvi H, et al. Questions and
263	challenges associated with studying the microbiome of the urinary tract. Ann Transl
264	<i>Med</i> 2017; 5 :33.
265	7-Brubaker L, Wolfe AJ. The female urinary microbiota, urinary health and common urinary
266	disorders. Ann Transl Med 2017;5:34.
267	8-Tang J. Microbiome in the urinary system—a review. AIMS Microbiol 2017;3 :143–54.
268	9-Whiteside SA, Razvi H, Dave S, Reid G, Burton JP. The microbiome of the urinary tract -
269	A role beyond infection. <i>Nat Rev Urol</i> 2015; 12 :81–90.
270	10- Nguyen-Hieu T, Khelaifia S, Aboudharam G, Drancourt M. Methanogenic archaea in

271 su	ıbgingival sites: A revi	iew. <i>Apmis</i> 2013; 121 :467–77.
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- 272 11- Grine G, Boualam MA, Drancourt M. Methanobrevibacter smithii, a methanogen
 273 consistently colonising the newborn stomach. *Eur J Clin Microbiol Infect Dis*274 2017;**32**:1–7.
- 275 12- Demonfort V, Henrissat B, Drancourt M. Archaea : Essential inhabitants of the human
 276 digestive microbiota. *Hum Microbiome J* 2017;**3**:1–8.
- 277 13- Drancourt M, Nkamga VD, Lakhe NA, Régis JM, Dufour H, Fournier PE, et al. Evidence
 278 of Archaeal Methanogens in Brain Abscess. *Clin Infect Dis* 2017;**65**:1–5.
- 14- Nkamga VD, Lotte R, Roger PM, Drancourt M, Ruimy R. Methanobrevibacter smithii
 and Bacteroides thetaiotaomicron cultivated from a chronic paravertebral muscle
 abscess. *Clin Microbiol Infect* 2016;**22**:1008–9.
- 15- Macario EC, Macario AJL. Methanogenic archaea in health and disease : A novel
 paradigm of microbial pathogenesis. *Int J Med Microbiol* 2009; **299**: 99–108.
- 284 16- Seng P, Drancourt M, Scola B La, Fournier P, Rolain JM. Ongoing Revolution in
- Bacteriology : Routine Identification of Bacteria by Matrix-Assisted Laser Desorption
 Ionization Time-of-Flight Mass Spectrometry. *Clin Infect Dis* 2010;49:543–51.
- 287 17- Grine G, Terrer E, Boualam MA, Aboudharam G, Chaudet H, Ruimy R, et al. Tobacco288 smoking-related prevalence of methanogens in the oral fluid microbiota. *Sci Rep*289 2018;8:91-7.
- 18- Raskin L, Stromley JM, Rittmann BE, Stahl D a. Group-Specific 16S Ribosomal-Rna
 Hybridization Probes To Describe Natural Communities of Methanogens. *Appl Environ Microbio*l 1994;60:1232–40.
- 293 19- Vianna ME, Conrads G, Gomes BPFA, Horz HP. T-RFLP-based mcrA gene analysis of

294	methanogenic archaea in association with oral infections and evidence of a novel
295	Methanobrevibacter phylotype. Oral Microbiol Immunol 2009;24:417-22.
296	20- Hungate RE, Macy J. The roll-tube method for cultivation of strict anaerobes. In:
297	Bulletins from the Ecological Research Committee 2012; 17: 123-6.
298	21- Khelaifia S, Raoult D, Drancourt M. A Versatile Medium for Cultivating Methanogenic
299	Archaea. <i>PLoS One</i> 2013;8(4).
300	22- La Scola B, Khelaifia S, Lagier JC, Raoult D. Aerobic culture of anaerobic bacteria using
301	antioxidants: a preliminary report. Eur J Clin Microbiol Infect Dis 2014;33:1781-3.
302	23- Nkamga VD, Huynh HTT, Aboudharam G, Ruimy R, Drancourt M. Diversity of Human-
303	Associated Methanobrevibacter smithii Isolates Revealed by Multispacer Sequence
304	Typing. <i>Curr Microbiol</i> 2015; 70 :810–5.
305	24- Dridi B, Henry M, El Khéchine A, Raoult D, Drancourt M. High prevalence of
306	Methanobrevibacter smithii and Methanosphaera stadtmanae detected in the human gut
307	using an improved DNA detection protocol. PLoS One 2009;4:25-9.
308	25- Million M, Tidjani Alou M, Khelaifia S, Bachar D, Lagier JC, Dione N, Brah S. Increased
309	Gut Redox and Depletion of Anaerobic and Methanogenic Prokaryotes in Severe Acute
310	Malnutrition. Sci Rep 2016;6: 1–11.
311	26- Huynh HTT, Pignoly M, Nkamga VD, Drancourt M. The Repertoire of Archaea
312	Cultivated from Severe Periodontitis. PLoS One 2015;4:8-10.
313	27- Bringuier A, Khelaifia S, Richet H, Aboudharam G, Drancourt M. Real-time PCR
314	quantification of Methanobrevibacter oralis in periodontitis. J Clin Microbiol
315	2013; 51 :993–4.
316	28- Faveri M, Feres M, Lc F, La G, Ja S. Prevalence and microbiological diversity of Archaea

- in peri-implantitis subjects by 16S ribosomal RNA clonal analysis. *J Periondont Res*2011;43:338–44.
- 29- Khelaifia S, Brunel JM, Raoult D, Drancourt M. Hydrophobicity of imidazole derivatives
 correlates with improved activity against human methanogenic archaea. *Int J Antimicrob Agents* 2013;41:544–7.
- 30- Khelaifia S, Lagier JC, Nkamga VD, Guilhot E, Drancourt M, Raoult D. Aerobic culture
 of methanogenic archaea without an external source of hydrogen. *Eur J Clin Microbiol Infect Dis* 2016;**35**:985–91.
- 31- Khelaifia S, Ramonet PY, Bedotto Buffet M, Drancourt M. A semi-automated protocol
 for Archaea DNA extraction from stools. *BMC Res Notes* 2013;6:186-9.
- 327 32- McKay LF, Holbrook WP, Eastwood MA. Methane and hydrogen production by human
 328 intestinal anaerobic bacteria. *Acta Pathol Microbiol Scand Ser B Microbiol* 329 2009;**90**:257–60.
- 330 33- Dridi B, Fardeau ML, Ollivier B, Raoult D, Drancourt M. The Antimicrobial Resistance
- 331 Pattern of Cultured Human Methanogens Reflects the Unique Phylogenetic Position of
- Archaea. J Antimicrob Chemother 2011;**66**: 2038–44.
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344 Author contributions

- GG and RL wrote the manuscript. GG, RL cultivated the methanogens and carried out the
- PCR. DC, RL and AC performed the clinical analyses. MD, DR and RR supervised the study.

347 **Conflicts of interests**

348 GG, RL, DC, AC, DR, MD, RR declare no potential conflict of interest.

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	Laboratory 1	Laboratory 2	Statistical analysis (+)
Number of patients	183	200	
Sex ratio (male/female)	56/127	93/107	p=0.0014 (Chi-square test)
Age	55 +/- 25	57 +/- 21	p=0.44 (t-test)
Number of urine samples	183	200	
Routine urine analysis			
pH*	6.36 +/- 0.97	6.31 +/- 0.97	p=0.62 (t-test)
Urine salinity ‰*	43.09 +/-18.06	42.18 +/- 16.86	p=0.61 (t-test)
White blood cells**	16.1 (0.1-3505)	8 (0-1000)	p=0.012 (t-test)
Red blood cells**	14.4 (0.1-7554)	7 (0-1000)	p=0.06 (t-test)
Standard culture +	53/183 (29%)	89/200 (45%)	p=0.002(Chi-square test)
Methanogen approach			
Archaea 16S rDNA PCR +	18/183 (10%)	16/200 (8%)	p=0.44(Chi-square test)
mcrA PCR +	18/183 (10%)	16/200 (8%)	p=0.44(Chi-square test)
Sequencing	M. smithii (n=18)	M. smithii (n=16)	
Methanogen culture +	15/183 (8.2%)	16/200 (8%)	p=0,83(Chi-square test)
Strain identification	M. smithii (n=15)	M. smithii (n=16)	

Table 1. Comparison of the data collected in laboratory 1 and in laboratory 2.

* pH and Urine salinity values are expressed as mean +/- SD.**White and red blood cells are expressed as median (range).

Type of UTI	Age (sex)	Underlying diseases of the genitourinary tract	Nosocomial	Leukocytes urine (/µL)	in Associated bacteria ** (CFU/mL)	Treatment (Duration)	Course of infection
Cystitis	87 (F)	No	Yes	>1000	Escherichia coli (10 ⁶)	Amoxicillin-clavulanic acid (7 days)	Favourable
	65 (F)	No	No	64	<i>Escherichia coli</i> (10 ⁶) and <i>Proteus mirabilis</i> (10 ⁶)	Fosfomycin-trometamol (1 day)	Reinfection at day 45 (<i>E. coli</i> and <i>P. mirabilis</i>)
	74 (F)	No	No	5	Klebsiella pneumoniae (10 ⁴)	Pivmecillinam (7 days)	Favourable
	55 (F)	No	No	592	Escherichia coli (10 ³)	Ofloxacin (NA)	Favourable
	25 (F)	No	No	140	Escherichia coli (10 ⁶)	Pivmecillinam (5days)	Favourable
	52 (F)	Renal transplant	Yes	10	Klebsiella pneumoniae (10 ⁶)	Ceftriaxone (NA)	Favourable
	50 (F)	No	No	464	Escherichia coli (10 ⁶)	NA	NA
	83* (F)	No	No	23	Escherichia coli (10 ⁶)	Fosfomycin and Colistin (42 days)	NA
	36 (F)	No	No	11	Escherichia coli (10 ⁶)	NA	NA
	20 (F)	No	No	78	Escherichia coli (10 ⁶)	Pivemecillinam (5days)	Favourable
	56* (M)	No	No	25	Escherichia coli (10 ⁶)	Ciprofloxacin (3 days)	Favourable

Table 2. Clinical and microbiological features of 19 patients presenting with UTIs and in whose urines *M. smithii* was detected

	59 (F)	Renal transplant	No	4	Klebsiella pneumoniae (10 ⁶)	Ciprofloxacin (7 days)	Favourable
	79* (F)	Chronic renal failure	No	85	Enterobacter cloacae (10 ⁶)	Amoxicillin-clavulanic acid (2 days)	Favourable
Pyelonephritis	49 (F)	Renal lithiasis	No	150	Enterobacter cloacae (10 ³)	Ceftriaxone IV and gentamicin (NA)	Favourable
	69 (M)	Renal lithiasis	No	20	Escherichia coli (10 ⁶)	Ceftriaxone IV (5days)	Favourable
	75 (F)	Vesico-vaginal fistula	No	>1000	Klebsiella pneumoniae (10 ⁶)	Imipenem IV (10 days)	Favourable
	83 (F)	No	No	53	Escherichia coli (10 ⁶)	Ofloxacin (1day) and amoxicillin-clavulanic acid (7days)	Favourable
Prostatitis	69 (M)	No	No	221	Escherichia coli (10 ⁵)	Ceftriaxone and gentamicin (NA)	Favourable
	50 (M)	No	No	>1000	Escherichia coli (10 ⁴)	Pivmecillinam (15 days)	Favourable

(*) *M. smithii* was detected in the urine of this patient using molecular methods, but not isolated in culture using the approach developed specifically for archaea. (**) Significant threshold was $\geq 10/\mu$ L for leukocyturia. NA: not available. Favourable: patient with favourable outcome based on clinical evidence.

Sample	spacer 1	spacer 2	spacer 3	spacer 4	spacer Type
13	Х	Х	Х		1
14	Х	Х	Х	Х	2
41		Х	Х		3
42		Х	Х		3
52	Х	Х	Х		1
58		Х	Х		3
63	Х	Х	Х	Х	2
75	Х	Х	Х	Х	2
99		Х	Х		3
106	Х	Х	Х		1
112		Х	Х		3
126	Х	Х	Х	Х	2
128	Х	Х	Х	Х	2
133	Х	Х	Х		1
149	Х	Х	Х	Х	2
167		Х	Х		3
4	Х	Х	Х	Х	2
6	Х	Х	Х		1
11	Х	Х	Х	Х	2
13		Х	Х		3
49	Х	Х	Х		1
67	Х	Х	Х	Х	2
81	Х	Х	Х	Х	2
87		Х	Х		3
108	Х	Х	Х	Х	2
114		Х	Х		3
115	Х	Х	Х		1
125		Х	Х		3
173	Х	Х	Х	Х	2
177	Х	Х	Х		1
188	Х	Х	Х	Х	2

Supplementary Table. Multispacer sequence typing (MST) genotyping of the 31 strains of *M. smithii* isolated during the study.