# The urinary microbiome in association with diabetes and diabetic kidney disease: A systematic review

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**Abstract:** The urinary microbiome, or urobiome, is a novel area of research that has been gaining attention recently. As urine was thought to be sterile for years, there is limited information about the composition of the urobiome in health and disease. The urobiome is reported affected by several factors and diseases such as diabetes, a disease that often leads to kidney damage. Thus, we need to understand the role of the urobiome to assess and monitor kidney disease related to diabetes over time. We conducted a systematic review to summarize knowledge about the urobiome in association with diabetes mellitus and diabetic kidney diseases. The search, conducted in PubMed and Web of Science electronic databases until July 2024, resulted in the selection of 18 studies including cross-sectional case-control studies, cross-sectional surveys and one prospective longitudinal study. In total, the urobiome of 1,571 people was sequenced, of which 662 people had diabetes, and of these 36 had confirmed diabetic kidney disease; 609 were healthy individuals, 179 had prediabetes or were at risk of type 2 diabetes mellitus and 121 did not have diabetes but had other comorbidities. Eight studies analysed data from females, one was focused on male data, and the other nine had mixed female-male data. Most of the studies had a small sample size, used voided midstream urine, and used 16S rRNA sequencing. This systematic review summarizes trends seen throughout published data available to have a first baseline knowledge of the urinary microbiome, and its microbiota, in association with diabetes.

**Keywords:** urinary microbiome; urinary microbiota; urobiome; albuminuria; diabetes; kidney disease

35 **1. Introduction**

36 Microbial communities living within us comprise viruses, bacteria, archaea, and small eukaryotes like
37 fungi and protists (1). These communities are symbiotic microbial networks that can perform vital
38 functions in the host and whose metabolites, or the lack of them, have influence in human health and
39 diseases (2–4). The intricate structure of these microbial ecosystems within their ecological niche,
40 their taxonomy and functional composition, are known as the microbiome (5). Similarly to our
41 fingerprints, each microbiome in the gut, vagina, skin or urinary tract are highly unique and they are
42 influenced by the environment, host genetics, lifestyle which are factors for which we have some
43 understanding; but there may be other factors influencing the microbiome for which we currently lack
44 understanding (4). Dysbiosis is a state where the microbiome composition or diversity are disrupted
45 or altered, or both, impeding normal functionality or causing low-grade inflammation that may play a
46 role in the onset and/or development of several diseases (5). Among the human microbiomes, the gut
47 microbiome is the best studied as it is seen as a "hidden" metabolic organ for human wellbeing
48 affecting immunity, neural, endocrinal or metabolic pathways (4,6). Gut dysbiosis has been related to
49 several diseases but if it is cause or effect of these diseases is a question that remains unclear (4,7,8).
50 While our understanding of the gut microbiome is evolving but limited, the knowledge about other
51 microbiomes is even more limited. This is particularly true for the urinary tract microbiome, or
52 urobiome, which was long believed to be sterile until molecular techniques revealed its presence
53 about a decade ago (9,10). The urobiome is characterized by a low biomass relative to the gut
54 microbiome, and its composition remains largely unexplored (9,11). Evidence suggests that the
55 urobiome differs between males and females. It is often associated with lactobacilli in females,
56 whereas in males is linked to *Corynebacterium* or *Staphylococcus* (9,11,12). The differences in the
57 urobiome between males and females have been attributed to the hormone oestrogen, which plays
58 a crucial role in glycogen production (11,13) and whose fluctuations with age and menopausal status
59 may affect the female urobiome (14). Bacteria of the genus *Lactobacillus* metabolize glycogen, and as
60 a byproduct of it there is lactic acid which acidifies the environment modulating the growth of other

61 bacteria (11). The acidic environment is assumed significant for the protective role that lactobacilli
62 play in both the vaginal and urinary microbiomes of women (9,13).
63 Diabetes, a disease characterized by elevated levels of glucose in the blood, is a leading cause of
64 cardiovascular disease, blindness or kidney damage (15). Diabetes, especially type 2 diabetes mellitus
65 (T2DM), has become a global health emergency whose prevalence have been rising for decades
66 affecting 537 million people globally in 2021, including 6.7 million related deaths (15,16). The
67 incidence of diagnosed diabetes in adults is stabilising in some high-income countries, but incidence
68 in children is still increasing (17–20). Impaired glycaemia contributes to enhanced risk of infections,
69 and it may explain why diabetic individuals have higher risk of urinary infections, pyelonephritis and
70 urosepsis (21). Not only individuals with diabetes have higher glucose, albumin and other proteins in
71 urine which may affect the urobiome composition, enhance bacterial growth of some species and may
72 influence the diversity of the urobiome; but also they have high glucose in urine which compromises
73 the immune response and uroepithelial integrity (21). However, we have limited information about
74 the composition of the urobiome related to diabetes. In addition, damage in the kidney blood vessels,
75 because of diabetes or other causes, may decline the estimated glomerular filtration rate (eGFR) which
76 impacts urinary functions as these organs are connected through the ureters to the urinary bladder
77 (22). Development of diabetic nephropathy may further turn in chronic kidney disease (CKD) or end
78 stage renal disease (ESRD), the former associated with diabetes but also other comorbidities while the
79 latter is mostly caused by diabetes (22).
80 As there is a clear knowledge gap regarding the role of the urobiome in diabetes and diabetic kidney
81 disease (DKD), the aim of this systematic review is to gather available data and synthetise the main
82 findings about the urobiome, particularly, the urinary microbiota in association with diabetes and
83 kidney conditions derived from diabetes such as CKD or ESRD.

84

85 **2. Materials and methods**

86 2.1 Search strategy

87 The comprehensive literature search was conducted according to the Preferred Reporting Items for
88 Systematic Reviews and Meta-analyses (PRISMA) guidelines (23). It was registered under accession
89 number of 565545 in the International Prospective Register of Systematic Reviews (PROSPERO) hosted
90 by the National Institute for Health and Care Research of the University of York (United Kingdom). A
91 comprehensive literature search on SCOPUS and PUBMED databases was conducted until July 2024.
92 We used the 'TITLE-ABS-KEY' search in SCOPUS (Web of Science) database while 'ALL FIELDS' were
93 used in PUBMED database. The search included the following terms [("urobiome" OR "urinary
94 microbiome" OR "urinary microbiota" OR "urinary tract microb*" OR "urine microb*" OR "urogenital
95 microbiome" OR "urogenital microbiota") AND ("kidney disease*" OR "chronic kidney disease" OR
96 "diabetes" OR "albuminuria" OR "diabetic nephropathy" OR "end stage renal disease*" OR "renal
97 disease*")] and it wanted to identify relevant clinical urinary microbiome studies associated with
98 diabetes and/or kidney impairment published from 1st January 2000 until 4th July 2024.

99 2.2 Study selection

100 We included studies that evaluated the urobiome sequencing any type of urine samples (urinary
101 bladder, urinary kidney or urogenital samples (24)) in relation with diabetes and conditions that affect
102 kidney functionality, and which can lead to chronic kidney disease or end stage renal disease. Studies
103 were excluded if they were review articles, editorials, comments, systematic reviews, books,
104 conference abstracts or any other type of article without full data available. The inclusion criteria
105 were: (i) analyses studying the bacterial species of urine; (ii) analyses of individuals with diabetes
106 and/or albuminuria and/or diabetic CKD and/or diabetic end-stage renal disease; (iii) human clinical
107 samples; and (iv) perform DNA sequencing through 16S ribosomal DNA gene or shotgun next
108 generation sequencing.

109 2.3 Data extraction

110 Throughout this synthesis, we used the PRISMA and PROSPERO frameworks and we incorporated the
111 associated guidance at each stage. Following the removal of duplicates, the first step was the
112 screening of studies by article type. After removal of non-suitable studies, the second step included a
113 screening of studies by title and/or abstract for relevance. Those that seemed relevant were the
114 preliminary selected publications and they were finally screened based on full-text information.
115 Studies lacking full-text availability or with missing or unclear information were excluded from the
116 evaluation (**Fig. 1**). Two independent authors (T.G and Y.T.L) screened studies by title and abstract
117 and, if discrepancies arose, they were discussed until they reached a common agreement. Preliminary
118 selected publications were fully screened by T.G., reviewed by Y.T.L, and if they fulfilled the inclusion
119 criteria, they were selected for this systematic review.

120 **Figure 1: Flow diagram of selected studies**. Diagram showing how studies in this systematic review about the urinary
121 microbiota and diabetes were selected.

122

123 **3. Results**

124 In total, eighteen studies were included including cross-sectional case-control studies, cross-sectional
125 surveys and one prospective longitudinal study (**Table 1** and **Supplementary Table 1**). These studies
126 altogether sequenced the urinary microbiome of 1,571 people, some participants were part of the
127 same cohort studied and were included multiple times (25–28). Of these individuals 662 had diabetes,
128 609 were healthy individuals, 179 had prediabetes or were at risk of diabetes, and 121 did not have
129 diabetes but had other comorbidities. Among the 662 diabetic individuals, 36 individuals had diabetic
130 kidney disease (29–31) while one studied CKD patients, most of them with diabetes, but did not
131 mention the primary cause of CKD (32). Sixteen of the studies used voided midstream urine samples,
132 or a modification of this collection technique, to avoid as much as possible skin or genitalia
133 microorganisms (**Supplementary Table 1**). The remaining two studies analysed urinary bladder
134 microbiota (catheterised urine) and, among these two, one was focused only on males (**Table 1**). Eight
135 studies included only female samples (four of these used the same cohort data); and nine studies had

136 mixed female-male data (**Table 1**). All studies except one used 16S sequencing which included twelve
137 studies with small sample sizes with less than 100 individuals, including five studies with less than 50
138 participants (**Table 1**). The only one that used shotgun sequencing had a small sample size (n = 29)
139 (**Supplementary Table 1**). Participants of these 18 studies were adults; individuals with diabetes
140 ranged between 50 and 72 years old on average, and healthy controls were similar in age but slightly
141 younger (if age was significantly different between groups, it is stated in the comments in **Table 1**). All
142 selected studies were carefully planned and to avoid bias they had strict exclusion criteria that are
143 explicitly detailed in **Supplementary Table 1**. Moreover, articles considered confounding factors such
144 as age or BMI and other relevant clinical factors, and if those were statistically significant between
145 compared groups, they are pointed up in the comments of **Table 1.**

146 Among the 11 case-control studies, 9 reported richness and α-diversity analyses of which three of the
147 studies observed a decrease in richness and α-diversity, one a decrease in richness, two a decrease in
148 α-diversity, one observed an increase in α-diversity but this study assessed kidney stones patients with
149 and without diabetes, and two studies did not observe differences in richness or α-diversity in the
150 urobiome of the individuals with diabetes compared to those without diabetes (**Table 1**). Among the
151 studies assessing only diabetic individuals, one study observed lower α-diversity in diabetic individuals
152 with pyuria, one study observed lower richness in patients with diabetic peripheral neuropathy, one
153 study did not observed differences in α-diversity when assessing urinary interleukin-8; and one study
154 observed increased richness in diabetic individuals compared to diabetic individuals with
155 hyperlipidaemia but decreased richness if compared to diabetic individuals with hypertension. Lastly,
156 the two studies assessing CKD reported a decrease of α-diversity as CKD worsened independently of
157 the primary cause of CDK (diabetes or other). Indexes calculated in each study are detailed in
158 **Supplementary Table 2** and the main ones are detailed in **Table 1.** Only seven studies reported β-
159 diversity analyses with variable results (**Table 1**); 4 reported differences in β-diversity, of which 2
160 described different urotypes (32–35); while 3 did not see differences in β-diversity(26,36,37).

161 Significant results in microbial species for diabetic adults (statistical significance set to $p < 0.05$) of each
162 article are summarised in **Table 1** and all details can be found in **Supplementary Table 3**. Comprising
163 all studies, most of the bacterial species were identified at the genus level; few at species level and
164 some at higher taxonomy levels. The relative abundance of 69 different bacteria was decreased, of 53
165 was increased in the urine of diabetic individuals while bacteria such as *Enterococcus,*
166 *Stenotrophomonas, Klebsiella* or Actinobacteriota had increased or decreased relative abundance
167 depending on studies (**Supplementary Table 3, Supplementary Table 4** and **Table 1**).

168 Overall, we could observe that the genus *Lactobacillus,* and at species level *Lactobacillus iners*, had
169 increased relative abundance in diabetic individuals in 6 and 2 studies, respectively; and it was related
170 to poor glycaemic control and with presence of interleukin-8 in urine (26,27,33–35,38). Three studies
171 reported a decrease in relative abundance of the family *Bifidobacteriaceae* in urine of individuals with
172 diabetes which includes the genera *Bifidobacterium* and *Gardnerella;* while one study reported the
173 absence of the genus *Gardnerella* in individuals with diabetes and hyperlipidemia *(*note: *Gardnerella*
174 is included now in the genus *Bifidobacterium*)(26,27,29,39). In addition, depletion of the family
175 *Peptoniphilaceae* in urine of T2DM patients was reported in several selected studies. In fact, studies
176 reported decreased relative abundance of this family being relevant genera *Peptoniphilus*,
177 *Anaerococcus, Finegoldia, Murdochiella* and *Gallicola*, which were reported decreased abundant in
178 four, three, two, one and one article, respectively (25,26,34,35). The genus *Parvimonas,* which belongs
179 to this family as well, was increased in relative abundance in diabetic individuals compared to non-
180 diabetics only in the elderly cohort (38); while its relative abundance was increased in the diabetic
181 cohort compared to the diabetic plus hypertension cohort (27). Lastly, three studies reported
182 increased relative abundance of *Escherichia* (*Escherichia-Shigella),* a well-known genus causing urinary
183 tract infections (UTI), which was related to poor glycaemic control and in two studies this genus was
184 related to urinary symptoms or pyuria in urine (even though individuals did not have an active UTI)
185 (31,34,40).

186 Taxonomic classification of bacteria identified in selected studies was updated according to Genome
187 Taxonomy Database release 220 (https://gtdb.ecogenomic.org/) at higher taxonomical levels (e.g.
188 phylum) but at lower levels was maintained to preserve clarity of the conclusions. For the most
189 accurate taxonomic annotation refer to details in **Supplementary Table 4**.

**Table 1:** Characteristics and results of selected studies. BMI = body mass index; CKD = chronic kidney disease; eGFR = estimated glomerular filtration rate; ESRD = end stage renal disease; FBG = fasting blood glucose; H = healthy controls; HbA1c = glycosylated haemoglobin; IL-8= interleukin 8; NS = Not stated; OTU = operational taxonomic unit; PD = peritoneal dialysis; SBP = systolic blood pressure; (TX)DM = (type X = 1 or X = 2) Diabetes mellitus; SD = standard deviation; vs = versus; * = studies that used data from participants/cohort of article 13 authored by Liu et al. 2017 (25); £ = sequencing of microbiota extracellular vesicles; ¥ = primers for phylum Firmicutes; and genera *Bacteroides* and *Bifidobacterium;* Ω = some or all individuals used antibiotic prophylaxis. ♀ = only females; ♂ = only males; ↓ = lower; ↑ = higher.

| Article | Coun-try | Total adult | Healthy vs diabetic adults | Age: mean ± SD | Context | Indexes results in diabetic individuals | Main findings and/or differences in relative abundance of diabetic individuals in urine | Other significant details or relevant information |
|---|---|---|---|---|---|---|---|---|
| Case-control studies with controls and diabetic individuals | | | | | | | | |
| (33) | China | 30 | 0 vs 15 | Kidney stones and T2DM: 56.20 ± 11.41<br><br>Kidney stones alone: 55.00 ± 11.69 | Urinary microbiota in the renal pelvis of patients with kidney stones plus T2DM and those with kidney stones alone | No differences in richness (Chao index)<br>↑ α - diversity (Shannon index)<br><br>β-diversity: the group with kidney stones alone and with kidney stones and T2DM clustered and could be differentiated | ↑*Sphingomonas*<br>↑*Propionibacterium*<br>↑ *Corynebacterium*<br>↑ *Cellulosimicrobium*<br>↑ *Methylophilus*<br>↑ *Lactobacillus*<br>↑ *Enhydrobacter*<br>↑ *Chryseobacterium*<br>↑ *Haemophilus*<br>↑ *Allobaculum* | The relative abundance of *Enhydrobacter, Chryseobacterium,* and *Allobaculum* genera exhibited correlations with fasting blood glucose and HbA1c values. |
| (41) | Italy | 75 | 25 vs 50 | H males: 62 ± 7<br><br>H females: 57 ± 6<br><br>T2DM males: 65 ± 9<br><br>T2DM females: 64 ± 10 | | No differences in richness or α - diversity (observed species, Chao, and Shannon indexes) | NS<br><br>[Statistical differences in prevalence (males and females): *Bifidobacterium breve, Corynebacterium pyroviciproducens, Lactobacillus gasseri* , and *Streptococcus agalactiae* were more prevalent in healthy controls. *Campylobacter ureolyticus, Corynebacterium coyleae, Corynebacterium glucuronolyticum, Enterococcus faecalis, Escherichia coli, Lactobacillus iners, Peptoniphilus grossensis,* and *Veillonella atypica* were more prevalent in T2DM individuals] | Total bacterial load and the abundance of total Bacillota were found to be elevated in patients with diabetes compared with the healthy control group, this was also true for females but not males. *Actinotignum schaalii, Bifidobacterium scardovii, Facklamia hominis, Negativicoccus succinicivorans,* and *Peptoniphilus lacrimalis* were less prevalent in T2DM males. *Aerococcus christensenii, Anaerococcus hydrogenalis, Brevibacterium ravenspurgense, Corynebacterium aurimucosum, Gardnerella vaginalis, Mobiluncus curtisii, Prevotella buccalis, Prevotella colorans*, and *Veillonella montpellierensis* were more prevalent in T2DM females whereas *Facklamia ignava* and *Winkia neuii* were less prevalent in T2DM females. |

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| (30) | China | 34 | 15 vs 19 | H: 43.73 ± 7.54<br><br>T2DM: 59.95 ± 9.38 | T2DM with diabetic kidney disease | ↓ α - diversity in T2DM (Shannon and Simpson indexes) | ↓Bacillota<br>↓ Bacteroidota<br>↑ Pseudomonadota<br>↑Acidobacteriota | Clinical characteristics between H and T2DM differed significantly in age; BMI; SBP; urea; uric acid; creatinine; natriuretic peptide; HbA1c (all ↓in H); and HDL and albumin (both ↑ in H). HbA1c was positively correlated with Acidobacteria and eGFR was positively correlated with Bacillota, Clostridiales, Clostridia, *Lactobacillaceae* and *Lactobacillus.* |
| (42) £ | South Korea | 691 | 368 vs 164 | 59.24 ± 6.13 (average of all groups and periods) | 4-year longitudinal study of sequencing extracellular microbial vesicles. Also, 164 prediabetics and 35 people transitioned | ↓ α - diversity (Shannon index) in all groups (possible effect of aging) | ↓ unclassified *Lachnospiraceae* GU174097_g | Low abundance of GU174097_g was a risk factor for T2DM development, and it was associated with T2DM progression. GU174097_g decreased ketone bodies levels; and thus reduced HbA1c levels. |
| (36) ♂ Ω | Czech Repu-blic | 58 ♂ | 0 vs 14 | 65.2 ± 13.8 (all) | Catheterized urine samples from men collected under anesthesia from the bladder prior to urological surgery. 14 individuals had diabetes (36 did not have diabetes and for 8 it was unknown) | ↓richness (OTUs, ACE and iChao2 indexes)<br><br>↓ α - diversity (Shannon and Simpson indexes)<br><br>β - diversity was no different | No statistically differences of specific taxa between T2DM and non-T2DM individuals | Lower richness was also associated with: (1) ≥75 years; (2) High cholesterol and/or hyperlipidemia; (3) antibiotic prophylaxis.<br>Smokers had higher α - diversity.<br>No significant differences in α - diversity for hypertension or CKD.<br><br>β – diversity was different between individuals with (1) CKD vs no CKD; (2) severe vs mild urinary symptoms; and (3) antibiotic prophylaxis vs without |
| (39) ¥ | Turkey | 60 | 15 vs 15 | H: 41.93 ± 11.3<br><br>Obese: 41.86 ± 8.53<br><br>Pre-DM: 49.6 ± 8.91<br><br>T2DM: 54 ± 7.76 | T2DM, prediabetic (n=15), obese (n=15) and H groups | NS | ↓ *Bifidobacterium* (same was observed for obese and prediabetics) | Clinical features statistically different: FBG between all groups; HbA1c between all groups except for H and obese individuals; BMI between all groups except for prediabetics and both obese and T2DM. Age was significantly lower in both H and obese vs T2DM individuals. Triglycerides and HDL were significantly lower in H vs both prediabetics and T2DM individuals |
| (35) ♀ | United States | 136 ♀ | 49 vs 87 | H: 50.5 ±10.6<br><br>T2DM: 51.4 ± 10.9 | Females with T2DM | β – diversity: Both H and T2DM clustered in 4-5 urotypes | ↑ *Lactobacillus*<br>↓ *Corynebacterium*<br>↓ *Staphylococcus* | H and T2DM groups differed significantly in FBG and HbA1c (both ↓in H). HbA1c was positively correlated with *Lactobacillus* and negatively associated with *Prevotella* |

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| | | | | | | Urotypes in T2DM:<br>*Lactobacillus* and *Enterobacteriaceae* vs H females: *Gardnerella* and mixed | [T2DM differences in presence/absence:<br>*Lactobacillus* was more present while *Corynebacterium, Anaerococcus, Finegoldia* and *Peptoniphilus* were less present] | and *Corynebacterium* in all cohort; and negatively associated with *Prevotella* also in the T2DM group. Prevalence of *Lactobacillus* increased with weight; and most of participants were obese. |
| (34) ♀ | China | 58 ♀ | 26 vs 32 | H: 57.62 ± 9.24<br><br>T2DM:<br>56.97 ± 8.01 | Females H and T2DM. T2DM microbiota were also assessed for lower urinary symptoms and levels of HbA1c | Richness and α – diversity were no different (OTUs, observed species, Chao, ACE, Shannon and Simpson indexes)<br><br>β - diversity<br>was different between H and T2DM | ↑ *Escherichia-Shigella*<br>↑ *Klebsiella*<br>↑ *Aerococcus*<br>↑*Delftia*<br>↑*Enterococcus*<br>↑*Alistipes*<br>↑*Stenotrophomonas*<br>↑ *Micrococcus*<br>↑ *Deinococcus*<br>↓ *Gallicola*<br>↓*Arcobacter*<br>↓*Arcanobacterium*<br>↓*Kocuria*<br>↓ *Murdochiella*<br>↓ *Solitalea*<br>↓ *Peptoniphilus* | FBG was ↑ in T2DM compared to H group.<br>HbA1c was ↑ in T2DM with urinary symptoms vs T2DM without.<br><br>The diabetic group with urinary symptoms (compared to the group without) had lower richness, different β – diversity; and:<br>↑*Escherichia-Shigella*<br>↑ *Campylobacter*<br>↑ *Megasphera*<br>↓ *Prevotella*<br>↓ *Dialister*<br>↓ *Anaerococcus*<br>↓ *Fusobacterium*<br>↓ *Prevotella_6,*<br>↓ *Mycoplasma*<br>↓ *Peptoniphilus*<br>↓ *Porphyromonas*<br>↓ *Fodinicola*<br>↓ *Brevundimonas*<br><br>The diabetic group with high HbA1c compared to the diabetic low HbA1c group had lower α – diversity, different β – diversity, and:<br>↑*Escherichia-Shigella*<br>↑ *Lactobacillus*<br>↓ *Prevotella*<br>↓ *Campylobacter*<br>↓ *Dialister*<br>↓ *Anaerococcus*<br>↓ *Peptoniphilus*<br>↓ *Porphyromonas*<br>↓ *Fodinicola* |
| (38) ♀ | China | 100 ♀ | 50 vs 50 | Elderly:<br>71.86 ± 6.70<br>Non-old:<br>50.06 ± 7.51 | Elderly vs non-elderly cohorts. Half of the females of each cohort had T2DM | ↓ richness<br>(OTU and Chao indexes) in elderly with T2DM | Elderly with T2DM (compared to elderly without):<br>↓ *Nitrospirae*<br>↓ *Odoribacter* | T2DM females treated with metformin. *Lactobacillus* had a decreased trend in T2DM patients with FBG > 10 mmol/l. |

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| | | | | | | No differences in α - diversity (Shannon and Simpson indexes) between the elderly with and without T2DM | ↓*Aeromonas*<br>↓*Clostridium*<br>↓*Enterobacter*<br>↓*Butyricimonas*<br>↓*Enterococcus*<br>↓ *Erwinia*<br>↓ *Fusobacterium*<br>↓ *Klebsiella*<br>↓ *Lachnobacterium*<br>↓*Stenotrophomonas*<br>↓*Phascolarctobacterium*<br>↑*Eggerthella*<br>↑ *Parvimonas*<br>↑*Bdellovibrio*<br>↑*Hydrogenophaga*<br>↑***Lactobacillus iners*** | *Lactobacillus iners* was not associated with FBG.<br>Elderly related differences: (1) relative abundance of Bacillota (Firmicutes) increased with BMI; (2) relative abundance of Bifidobacteria was negatively correlated with age; (3) *Lactobacillus* decreased with age but was not associated with pH; (4) ↑ abundance of *Sphingobium* and *Bosea;* and (5) ↓ abundance of *Sneathia; Geobacillus; Shuttleworthia; Bacillus; Gemella; Bdellovibrio; Hydrogenophaga; Proteus; Novosphingobium.* |
| (25) ♀ | China | 140 ♀ | 70 vs 70 | Matched H and T2DM individuals.<br><br>70% > 56 years or older; only 8% younger than 45 years | Authors matched individual characteristics between the H and the T2DM groups | ↓ richness<br>(number of OTUs, observed species, ACE and Chao1 indexes)<br><br>↓ α-diversity (Shannon index) | ↑ Actinobacteria<br>↑ *Porphyromonas*<br>↑ *Flavobacteriales*<br>↑ *Collinsella*<br>↓ *Akkermansia muciniphila*<br>↓ Bacteroidota<br>↓ *Prevotellaceae*<br>↓ *Prevotella*<br>↓*Pseudomonadales*<br>↓*Peptoniphilus*<br>↓ *Citrobacter*<br>↓Actinobacteriota<br>↓ *Synergistota*<br>↓*Acinetobacter*<br>↓*Campylobacter*<br>↓*Campylobacterales*<br>↓ *Staphylococcus*<br>↓ *Anaerococcus*<br>↓ *Halomonas*<br>↓ *Finegoldia*<br>↓ *Streptococcus*<br>↓*Veillonella*<br>↓*Clostridium*<br>↓*Corynebacterium* | Actinobacteria increase was associated with higher BMI; increased FBG and urine glucose.<br><br>*Akkermansia muciniphila* decrease was associated with FBG and urine glucose.<br><br>Urine glucose, FBG, urine infections, hypertension and hyperlipidemia were statistically ↑ in T2DM compared to H group.<br><br>Authors studied hypertension and hyperlipidemia in article by (27); inflammatory markers in article by (Ling et al. 2017) and the effect on diet in article 7 (28). |
| (31) Ω | United States | 29 | 8 vs 6 | H: 34.57<br><br>ESDR T1DM: 51.00 | Transplant ESRD patients. Kidney damage caused by 4 primary diagnoses: other, T1DM, T2DM, and hypertension. | ↓ α-diversity (Shannon and inverse Simpson indexes)<br><br>↓ richness (observed species) | ↑ *Enterococcus faecalis*<br>↑ *Enterococcus faecium*<br>↑ *Enterococcus sp.*<br>↑ *Escherichia coli*<br>↑ *Escherichia sp.*<br>↓ *Cutibacterium acnes* | ↑ Bacillota and ↓Actinobacteria because *Enterococcus* belongs to the former phylum while as *Mobiluncus* belongs to the latter.<br>Both groups had similar folate metabolism (which is the target of |

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| | | | | ESDR T2DM: 64.33<br><br>(all ESRD: 53.28) | 15 individuals had non-diabetic ESRD. Trimethoprim - sulfamethoxazole was given prophylactically | β-diversity: primary diagnoses had no significant differences in bacterial composition; but all had with the H | ↓ *Corynebacterium*<br>↓*Mobiluncus curtisii* | trimethoprim -sulfamethoxazole) but had differences in specific enzymes. Dihydrofolate synthase/folypolygluta-mate synthase was increased in transplant groups. |
| Studies with diabetic individuals only | | | | | | | | |
| (40) | | Taiwan | 7 | 0 vs 7 | T2DM No-pyuria: 59 ± 5.7<br><br>T2DM Pyuria: 59.66 ± 6.5 | All 7 individuals with T2DM and SGLT2 therapy; 3 with pyuria in urine; 4 without | ↓ α - diversity in T2DM with pyuria compared with T2DM without pyuria (Shannon index) | ↑*Escherichia-Shigella* in individuals with pyuria | 7 T2DM individuals with negative standard urine cultures. eGFR stage II (60–89 mL/min) mildly impaired kidney function |
| (37) ♀ | | China | 30 ♀ | 0 vs 30 | T2DM with diabetic peripheral neuropathy: 55.82 ± 7.83<br><br>T2DM only: 54.85 ± 7.49 | Among the 30 females with T2DM, 17 females also had diabetic peripheral neuropathy | ↓richness in DPN group (OTUs, observed species, ACE and iChao2 indexes)<br><br>α – diversity was no different (Shannon and Simpson indexes)<br><br>β – diversity was no different | Group with DPN:<br>↑ *Mycoplasmataceae*<br>↓Propionibacteriaceae<br>↓ Pseudobdellovibrioinaceae<br>↓Sphingobacteriaceae | T2DM females: 17 with DPN and 13 without.<br><br>Both groups did not differ in clinical characteristics. |
| * ♀ | (27 ) | China | 70 ♀ | 0 vs 70 | T2DM: 56.28 ± 13.91<br><br>T2DM + hypertension: 70.42 ± 9.00<br><br>T2DM + hyperlipidemia: 54.43 ± 10.66<br><br>T2DM + both: 69.81 ± 9.64 | Only analysis of the T2DM individuals from article 13 that had different clinical features. The T2DM cohort was divided: 28 had T2DM; 24 had T2DM + hypertension; 7 had T2DM + hyperlipidaemia; 11 had T2DM and both hypertension + hyperlipidaemia | ↑ richness compared to the hyperlipidemia only group (number of reads and number of OTUs)<br><br>↓ richness compared to hypertension groups (number of reads and number of OTUs)<br><br>No differences in α - diversity (Shannon and Simpson indexes) between the groups | <u>Compared to T2DM + hypertension group, the group **with only** T2DM had:</u><br>↑ *Lactobacillus iners*<br>↑ *Acinetobacter rhizosphaerae*<br>↑ *Acinetobacter schindleri*<br>↑*Lactobacillus*<br>↑*Enterobacter*<br>↑*Klebsiella*<br>↑*Shuttleworthia*<br>↑*Sneathia*<br>↑ *Parvimonas*<br>↑*Megasphera*<br>↑*Erwinia*<br>↓ *Kocuria palustris*<br>↓ *Aeromonas*<br>↓ *Roseburia*<br>↓ *Ruminococcus*<br><br><u>Compared to T2DM + hyperlipidemia, the group **with only** T2DM had:</u><br>↓ *Faecalibacterium*<br>↓ *Collinsella* | The T2DM cohort was divided: 28 T2DM; 24 T2DM + hypertension; 7 T2DM + hyperlipidemia; 11 T2DM + hypertension + hyperlipidemia. The duration of T2DM was significantly shorter in the two groups without hypertension.<br><br>The four most abundant bacteria in all groups were Proteobacteria, Bacillota, Bacteroidota and Actinobacteria. The fifth varied from Fusobacteria (inT2DM and T2DM with both conditions), Synergistota (group with hypertension) and Acidobacteria (group with hyperlipidemia).<br><br>Predominant genera: in T2DM *Lactobacillus, Prevotella,* and *Acinetobacter;* in T2DM + hyperlipidemia *Lactobacillus, Prevotella* and *Halomonas*; in T2DM + hypertension *Prevotella, Streptococcus*, and *Bacteroides*; and in T2DM + hypertension + |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | ↓ *Oscillospira* ( diagnostic factor to differentiate both groups)<br><br><u>Compared to T2DM + hypertension and hyperlipidemia, the group **with only** T2DM had:</u><br>↑*Acinetobacter rhizosphaerae*<br>↑ *Shuttleworthia*<br>↓*Gemella*<br>↓*Prevotella*<br><br><u>Compared to T2DM + hypertension and hyperlipidemia, the group with T2DM + hyperlipidemia had:</u><br>↑*Acinetobacter rhizosphaerae*<br>↑*Faecalibacterium*<br><br><u>Compared to T2DM + hypertension, the group with T2DM + hyperlipidemia had:</u><br>↑ *Klebsiella*<br>↑ *Novosphingobium*<br>↑ *Lactobacillus*<br>↑ *Enterobacter*<br><br><u>The two groups with hypertension did not show differences</u> | hyperlipidemia *Prevotella, Lactobacillus* and *Bacillus*.<br><br>The groups with hypertension had presence of *Eggerthella lenta* but this species was absent in the group with only diabetes; while *Gardnerella* was absent in the hyperlipidemia group but it was present in the other groups.<br><br>Several bacteria correlated with FBG, blood pressure and lipidic profiles. *Atopobium* was positively correlated with FBG in the T2DM group. *Allobaculum* and *Odoribacter* were negatively correlated with diastolic pressure in the hypertension and hypertension plus hyperlipidemia groups, respectively. *Rikenella* and *Dorea* were positively correlated with triglycerides in the hyperlipidemia group and with LDL in the hypertension plus hyperlipidemia group, respectively. |
| * ♀ | (26 ) | China | 70 ♀ | 0 vs 70 | T2DM with IL-8 in urine:<br>65.78 ± 13.58<br><br>T2DM without IL-8 in urine:<br>59.25 ± 11.06 | T2DM females whose levels of IL-8 were evaluated. IL-8 is a potential biomarker for diagnosing of urinary infections | α – diversity was no different between T2DM females with and without IL-8 in urine (Shannon and Simpson indexes)<br><br>β-diversity: the group with Il-8 and without IL-8 in urine clustered together and could be differentiated | T2DM females without IL-8:<br>↓*Bifidobacteriaceae*<br>↓*Shuttleworthia*<br>↓ *Thermales*<br>↓ *Streptococcus anginosus*<br>↓ *Acinetobacter rhizosphaerae*<br>↓ *Acinetobacter schindleri*<br>↓ *Lactobacillus iners*<br>↓ *Akkermansia muciniphila*<br>↓ *Mobiluncus*<br>↓ *Peptoniphilus*<br>↓ *Corynebacterium*<br>↓ *Gemella*<br>↓ *Enterococcus*<br>↓ *Geobacillus*<br>↑ *Prevotella copri* | *Ruminococcus* was positively associated with urinary IL-8. Urinary IL-8 was high in T2DM patients with ↑ *Pseudomonas* and ↑ *Klebsiella*.<br><br>Clinical features between the T2DM with IL-8 and without IL-8 in urine differed in age, BMI and pH, white blood cells, leukocyte esterase, protein, glucose, and nitrites in urine ( all characteristics ↑ in the group with IL-8) |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | ↑ *Prevotella stercorea*<br>↑*Bacteroides uniformis*<br>↑*Coprococcus eutactus*<br>↑ *Faecalibacterium*<br>↑ *Megamonas*<br>↑ *Comamonas*<br>↑*Pseudomonas*<br>↑*Phascolarctobacterium* | |
| * ♀ | (28 ) | China | 140 ♀ | 70 vs 70 | Matched H and T2DM individuals.<br><br>70% > 56 years or older; only 8% younger than 45 years | T2DM association with urinary IL-8 and evaluation of the effect of diet on urinary microbiota | NS | NS | *Ruminococcus* was positively associated with urinary IL-8 (results from Ling et al. 2017). Water weakened the positive relationship between *Ruminococcus* and IL-8 in urine while fiber, vitamin B3 and vitamin E enhanced the positive relationship. |
| Studies with individuals that have kidney or renal disease | | | | | | | | | |
| (29) | | Portu-gal | 46 | 0 vs 14 | 56.9 ± 10.7 | All individuals with CKD and on peritoneal dialysis; the rest had other comorbidities | ↓ α - diversity (Shannon index) as CKD-PD worsened (independently of the cause) | ↓*Atopobium*<br>↓ *Dermabacter*<br>↓*Gardnerella* | Apart from diabetes in CKD patients, significance was found for sex: *Lactobacillus* linked to females; *Staphylococcus* and *Anaerococcus* to males. |
| (32) | | United States | 77 | 23 vs 54 | Males: 76.6 ± 7.6<br>Females:<br>70.5 ± 8.2 | All individuals with CKD but no dialysis (70% of them with diabetes) | ↓ α - diversity (Shannon index) as CKD worsened (independently of cause). Richness and α – diversity were no different between diabetic and non-diabetic CKD individuals (Chao; Inverse Simpson and Shannon indexes). | NS | Richness and α – diversity were no different by age or obesity status. β – diversity: Different urotypes.<br><br>Urinary incontinence and ↑ eGFR was associated with ↑ α-diversity. |

196 **4. Discussion**

197 This study has assessed the urinary microbiota related to diabetes and diabetes-related kidney
198 diseases through a systematic review. This work has summarised statistically significant results
199 reported by selected studies with a threshold of $p \leq 0.05$. The main findings of this study were: (1) a
200 decreased richness and α-diversity in urinary microbiota in individuals with diabetes compared to
201 healthy controls; (2) α-diversity was not different within diabetic individuals, except if they showed
202 urinary symptoms (e.g. urgency to pee) although they did not have a (diagnosed) urinary infection; (3)
203 α-diversity decreases with the evolution of kidney disease independently of the primary cause (e.g.
204 kidney disease primarily caused by diabetes or hypertension or other pathology); (4) β-diversity
205 urinary microbiota composition between healthy controls and diabetic individuals results were
206 conflicting between studies and may depend on the stage of the disease and/or the grade of kidney
207 damage; (5) patients with T2DM shared the depletion of *Peptoniphilus, Anaerococcus, Finegoldia* and
208 *Gallicola* (all formerly part of the genus *Peptostreptococcus* and inside the *Peptoniphilaceae* family),
209 or *Lachnospiraceae* that are butyrate-producing bacteria; (6) patients with diabetes shared the
210 enrichment of pro-inflammatory microbes such as Pseudomonadota (formerly Proteobacteria) and of
211 bacteria of order Lactobacillales such as *Lactobacillus* or *Enterococcus*; (7) cohort sizes were small
212 (from a few samples to more than one hundred) and the only one study that was large (nearly seven
213 hundred samples) had data restricted to extracellular microbial vesicles; (8) except in two studies,
214 voided midstream urine samples were analysed and those are often referred as urogenital samples
215 due to likely cross-contamination with skin or genitalia microbiota during collection; and, (9) although
216 data showed similar trends, some bacterial phyla, families, and genera had controversial results,
217 probably due to the lack of resolution of these studies using 16S sequencing.

218 One of the main findings of this systematic review is the depletion of the family *Peptoniphilaceae* in
219 urine of T2DM patients as a trend. The most relevant genera of this family for the urobiome being
220 *Peptoniphilus*, *Anaerococcus, Finegoldia, Murdochiella, Parvimonas* and *Gallicola*. These genera are
221 gram-positive obligate anaerobic cocci that have been described as producers of short-chain fatty

222 acids such as butyrate (*Peptoniphilus*, *Anaerococcus,* and *Gallicola*) and acetate (*Peptoniphilus,*
223 *Parvimonnas, Gallicola* and *Finegoldia)* or lactate *(Murdochiella)* (43). *Parvimonas* increased in relative
224 abundance in diabetic individuals compared to non-diabetics only in the elderly cohort (38); while its
225 relative abundance was increased in the diabetic cohort compared to the diabetic plus hypertension
226 cohort (27). In the latter, if diabetes is accompanied by other factors such as hypertension, there is
227 decreased abundance of this genus pointing to a depletion as the health status worsens. *Peptoniphilus*
228 and *Anaerococcus* abundance may be associated to glycosylated haemoglobin HbA1c as diabetic
229 individuals with high HbA1c had decreased relative abundance of these two genera in urine compared
230 to diabetic individuals with low HbA1c (34).

231 The genus *Lactobacillus* seems to be more abundant in the urine of diabetic individuals according to
232 this systematic review. *Lactobacillus iners*, which has been described as a transitional species that
233 colonizes the vagina after an ecological disturbance (44), was found enriched in the urine of T2DM
234 females (27,38) and in the same cohort with higher levels of inflammatory interleukin-8 in urine (26).
235 Other species of this genus such as *Lactobacillus crispatus* and *Lactobacillus gasseri* have been
236 associated with lack of lower urinary tract symptoms and lack of urgency urinary incontinence,
237 respectively; and levels of *Lactobacillus* in the vagina are reported to be associated with menopausal
238 status and with reduced risk of UTI (35). Although lactobacilli have generally been considered
239 beneficial and the normal microbiota of the urobiome and vagina, there is increasing evidence that
240 some *Lactobacillus* spp. may be pathogenic or related to urinary symptoms (45,46). The association
241 between the different species of *Lactobacillus* and their role in T2DM needs further investigation,
242 considering that age, oestrogen, HbA1c and/or BMI could also be important factors affecting the
243 abundance of this genus. In this context, HbA1c was positively correlated with *Lactobacillus.*
244 Individuals with T2DM and high HbA1c had higher abundance of this genus compared to those with
245 T2DM and low HbA1c levels (34,35). Another point to consider is that the genus *Lactobacillus* is
246 naturally resistant to widely used antibiotics and T2DM individuals often have multiple infections that

247 require the use of them (34). Thus, we could hypothesize that antibiotic therapy may imbalance the
248 urobiome allowing *Lactobacillus* to grow excessively which may induce inflammation.

249 Diabetic, prediabetic and obese individuals had lower abundance of the genus *Bifidobacterium* in
250 urine; and the abundance of this genus was negatively correlated with age (38,39). In this line, T2DM
251 patients with hyperlipidemia lacked *Bifidobacterium* (former *Gardnerella*) (27). This was also true for
252 the former genus *Gardnerella,* that is now included in the genus *Bifidobacterium,* that have decreased
253 abundance in CKD patients with diabetes (29). *Bifidobacterium* (*Gardnerella*) was a common urotype
254 in healthy females compared to diabetic females that had *Lactobacillus* or *Enterobacteriaceae*
255 urotypes (35). *Bifidobacteriaceae* had decreased relative abundance in diabetic females without
256 interleukin-8 in urine in the study by Ling et al; but this group had also lower age and BMI compared
257 to the group with interleukin-8 (26). These results may imply that diabetes but also age, BMI or levels
258 of interleukin-8 could influence the relative abundance of *Bifidobacterium* in urine (26).

259 The role of the genus *Prevotella* in the urinary microbiota is unclear. Although HbA1c was negatively
260 correlated with *Prevotella* in T2DM patients (35) and Chen et al. reported a decrease of this genus in
261 individuals with diabetes with high urinary symptoms (34), other studies have found that this genus
262 was more prevalent in T2DM (41) or that urotypes and enrichment of *Prevotella* was associated with
263 urinary incontinence patients (12,47). Therefore, similarly to findings with the genus *Lactobacillus,* we
264 will likely need higher resolution studies, preferably shotgun metagenomics, to identify which species
265 or strains of these genera may play different roles in the urobiome.

266 UTIs are common in diabetic patients and are associated with poor glycaemic control, which
267 compromises kidney function and impairs quality of life (48). That is why many selected articles also
268 examined urinary symptoms, as hospitalization for UTIs caused by diabetes is more than twice as
269 common as hospitalizations for UTIs caused by other factors. The use of sodium–glucose cotransporter
270 2 inhibitors as therapy for diabetes may contribute as these drugs reduce glucose reabsorption, thus
271 lowering the level on blood, but causing glycosuria in urine which could increase the risk of UTIs in

272 those patients (49). Hyperglycaemia and diabetes has been shown to reduce psoriasin and occludins
273 levels in the urinary tract leading to be more prone to urinary tract infections (21). Moreover, psoriasin
274 is an antimicrobial peptide with a broad protective role against *Escherichia coli* and *Enterococcus*
275 urinary infections, two of the most common pathogens causing UTI in diabetics (21). In addition, low-
276 grade inflammation with higher concentration of proinflammatory cytokines and the depletion of
277 producers of short-chained fatty acids such as butyrate, which has beneficial effects on the host (50),
278 may affect mucosa integrity, bacterial translocation and host–urine microbiota interactions in T2DM
279 patients.

280 The results of this systematic review have also highlighted that urinary microbiota diversity seems
281 lower in CKD or ESRD and it drops as the disease progresses independently of the primary cause
282 (29,32). Diabetes progression to kidney damage reduces eGFR and as eGFR declines the excretion of
283 uromodulin follows the same trend. Uromodulin, an urinary antimicrobial peptide whose function is
284 to stick to bacteria forming larger particles which enhance their excretion by the kidney, is produced
285 exclusively in the renal tubules and is affected by the decline of renal function (32) which may also
286 influence bacterial growth, microbiota diversity and risk of UTI.

287 Generally, adults with diabetes show lower abundance of short-chain fatty acids producing bacteria
288 in the gut compared to healthy controls (4). This systematic review reveals that diabetic individuals
289 have similar trends but also differences in their urinary microbiota. Lower relative abundance of short-
290 fatty acid butyrate-producing bacteria of the family *Peptoniphilaceae* and a higher relative abundance
291 of *Lactobacillus* was a common trend of this systematic review.

292 <u>Strengths and limitations</u>

293 To our knowledge, this review represents the first comprehensive analysis of the current
294 understanding of the urobiome, specifically the urinary microbiota, in the context of diabetes. One
295 notable strength of this review is the identification of emerging trends within the urobiome across
296 multiple studies. For instance, this is the first article to highlight the significance of the

297 *Peptoniphilaceae* family within the urobiome of diabetic individuals, which has frequently been
298 reported as less prevalent or reduced in relative abundance in urine of diabetic individuals.
299 Additionally, this work is strengthened by its systematic approach, adhering to PRISMA and PROSPERO
300 guidelines, ensuring standardized and reproducible results.

301 However, we also recognize some limitations. First, we were unable to perform meta-analyses due to
302 the considerable heterogeneity of the included studies. Second, while two studies analysed
303 catheterized urine, reflecting the microbiota of the bladder or renal pelvis, most of the studies relied
304 in voided midstream collection techniques that may also capture microbiota from the genitalia or skin
305 (Linda Brubaker et al., 2021). Moreover, since most studies employed 16S sequencing, the resolution
306 of the results is limited. This limitation means that the same genera identified across different studies
307 could refer to distinct bacterial species, potentially explaining some discrepancies in the findings.
308 Another limitation is the evolving taxonomical classification of bacteria. Taxonomic reclassification
309 may have occurred since the original articles were published. Where possible, we have updated
310 higher-level classifications (e.g., from Firmicutes to Bacillota) while maintaining the original
311 nomenclature at lower levels to preserve the clarity of the conclusions (e.g., Gardnerella instead of
312 Bifidobacterium). For the most accurate taxonomic annotation, we refer readers to the Genome
313 Taxonomy Database release 220 (https://gtdb.ecogenomic.org/), with detailed information provided
314 in **Supplementary Table 4**.

315 **5. Future perspectives**

316 There are significant knowledge gaps regarding the role and impact of the urobiome in both health
317 and disease. This an emerging field that has attracted increasing attention in recent years, but the
318 influence of the urobiome on conditions such as diabetes and diabetic kidney disease remains largely
319 unexplored. While this systematic review has identified common trends across studies—such as the
320 depletion of *Peptoniphilaceae* and the increased abundance of *Lactobacillus* in the urine of diabetic

adults—the evidence remains insufficient due to the small sample sizes of most studies and the limited resolution provided by 16S sequencing.

To advance urobiome research, large-scale population-based studies are urgently needed, along with the application of higher-resolution techniques like shotgun metagenomics to more precisely characterize the urinary microbiome. Such approaches could help identify specific microbial species that play critical roles in maintaining a healthy urobiome, as well as those involved in the onset and progression of diseases such as diabetes and conditions affecting kidney function.

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**References:**


1. Ramirez J, Guarner F, Bustos Fernandez L, Maruy A, Sdepanian VL, Cohen H. Antibiotics as Major Disruptors of Gut Microbiota. Front Cell Infect Microbiol. 2020 Nov 24;10:572912.

2. Kirby TO, Hendrix EK, Ochoa-Repáraz J. The gut microbiota as a therapeutic approach for obesity. In: Microbiome and Metabolome in Diagnosis, Therapy, and other Strategic Applications. Elsevier; 2019. p. 227–34.

3. Aasmets O, Krigul KL, Lüll K, Metspalu A, Org E. Gut metagenome associations with extensive digital health data in a volunteer-based Estonian microbiome cohort. Nat Commun. 2022;13(1):1–11.

4. Fan Y, Pedersen O. Gut microbiota in human metabolic health and disease. Nat Rev Microbiol. 2021 Jan 4;19(1):55–71.

5. Berg G, Rybakova D, Fischer D, Cernava T, Vergès MCC, Charles T, et al. Microbiome definition re-visited: old concepts and new challenges. Microbiome. 2020 Jun 30;8(1):1–22.

6. Guinane CM, Cotter PD. Role of the gut microbiota in health and chronic gastrointestinal disease: understanding a hidden metabolic organ. Ther Adv Gastroenterol. 2013;6(4):295.

7. Stolfi C, Maresca C, Monteleone G, Laudisi F. Implication of Intestinal Barrier Dysfunction in Gut Dysbiosis and Diseases. Biomedicines. 2022 Jan 27;10(2):289.

8. Donaldson GP, Lee SM, Mazmanian SK. Gut biogeography of the bacterial microbiota. Nat Rev Microbiol. 2016 Jan;14(1):20–32.

9. Roth RS, Liden M, Huttner A. The urobiome in men and women: a clinical review. Clin Microbiol Infect. 2022;▪:1.

10. Hilt EE, McKinley K, Pearce MM, Rosenfeld AB, Zilliox MJ, Mueller ER, et al. Urine Is Not Sterile: Use of Enhanced Urine Culture Techniques To Detect Resident Bacterial Flora in the Adult Female Bladder. Munson E, editor. J Clin Microbiol. 2014 Mar;52(3):871–6.

11. Brubaker L, Putonti C, Dong Q, Wolfe AJ. The human urobiome. Mamm Genome. 2021 Aug;32(4):232–8.

12. Carnes MU, Siddiqui NY, Karstens L, Gantz MG, Dinwiddie DL, Sung VW, et al. Urinary microbiome community types associated with urinary incontinence severity in women. Am J Obstet Gynecol. 2024 Mar;230(3):344.e1-344.e20.

13. Nunn KL, Forney LJ. Unraveling the Dynamics of the Human Vaginal Microbiome. Yale J Biol Med. 2016 Sep;89(3):331–7.

14. Hugenholtz F, Van Der Veer C, Terpstra ML, Borgdorff H, Van Houdt R, Bruisten S, et al. Urine and vaginal microbiota compositions of postmenopausal and premenopausal women differ regardless of recurrent urinary tract infection and renal transplant status. Sci Rep. 2022 Feb 17;12(1):2698.

15. Reed J, Bain S, Kanamarlapudi V. A Review of Current Trends with Type 2 Diabetes Epidemiology, Aetiology, Pathogenesis, Treatments and Future Perspectives. Diabetes Metab Syndr Obes Targets Ther. 2021 Aug;Volume 14:3567–602.

16. Magliano D, Boyko EJ. IDF diabetes atlas. 10th edition. Brussels: International Diabetes Federation; 2021.

17. Magliano DJ, Chen L, Islam RM, Carstensen B, Gregg EW, Pavkov ME, et al. Trends in the incidence of diagnosed diabetes: a multicountry analysis of aggregate data from 22 million diagnoses in high-income and middle-income settings. Lancet Diabetes Endocrinol. 2021 Apr;9(4):203–11.

18. Zhang K, Kan C, Han F, Zhang J, Ding C, Guo Z, et al. Global, Regional, and National Epidemiology of Diabetes in Children From 1990 to 2019. JAMA Pediatr. 2023 Aug 1;177(8):837.

19. Nagl K, Waldhör T, Hofer SE, Blauensteiner N, Fritsch M, Fröhlich-Reiterer E, et al. Ongoing Increase in Incidence of Diabetes in Austrian Children and Adolescents (1989–2021): Results from a Nationwide Registry. Habeb AM, editor. Pediatr Diabetes. 2023 Aug 18;2023:1–7.

20. Sun H, Saeedi P, Karuranga S, Pinkepank M, Ogurtsova K, Duncan BB, et al. IDF Diabetes Atlas: Global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045. Diabetes Res Clin Pract. 2022 Jan;183:109119.

21. Mohanty S, Kamolvit W, Scheffschick A, Björklund A, Tovi J, Espinosa A, et al. Diabetes downregulates the antimicrobial peptide psoriasin and increases E. coli burden in the urinary bladder. Nat Commun. 2022 Sep 20;13(1):4983.

22. Natesan V, Kim SJ. Diabetic Nephropathy - a Review of Risk Factors, Progression, Mechanism, and Dietary Management. Biomol Ther. 2021 Jul 1;29(4):365–72.

23. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ. 2021 Mar 29;n71.

24. Brubaker L, Gourdine JPF, Siddiqui NY, Holland A, Halverson T, Limeria R, et al. Forming Consensus To Advance Urobiome Research. mSystems. 2021;6(4).

25. Liu F, Ling Z, Xiao Y, Lv L, Yang Q, Wang B, et al. Dysbiosis of urinary microbiota is positively correlated with Type 2 diabetes mellitus [Internet]. Vol. 8, Oncotarget. 2017 p. 3798–810. Available from: www.impactjournals.com/oncotarget/

26. Ling Z, Liu F, Shao L, Cheng Y, Li L. Dysbiosis of the urinary microbiota associated with urine levels of proinflammatory chemokine interleukin-8 in female type 2 diabetic patients. Front Immunol. 2017 Aug 25;8(AUG).

27. Liu F, Ling Z, Xiao Y, Yang Q, Wang B, Zheng L, et al. Alterations of urinary microbiota in type 2 diabetes mellitus with hypertension and/or hyperlipidemia. Front Physiol. 2017 Mar 3;8(MAR).

28. Liu F, Ling Z, Tang C, Yi F, Chen YQ. Moderation effects of food intake on the relationship between urinary microbiota and urinary interleukin-8 in female type 2 diabetic patients. PeerJ. 2020;2020(1).

29. Araujo R, Merino-Ribas A, Pereira L, Campos J, Silva N, Alencastre IS, et al. The urogenital microbiome in chronic kidney disease patients on peritoneal dialysis. Nefrología. 2023 Aug;

30. Yang Y, Ma C, Li S, Cai W, Dai W, Zhang X, et al. Urinary microbiota and serum metabolite analysis in patients with diabetic kidney disease. Heliyon. 2023 Aug 1;9(8).

31. Rani A, Ranjan R, McGee HS, Andropolis KE, Panchal DV, Hajjiri Z, et al. Urinary microbiome of kidney transplant patients reveals dysbiosis with potential for antibiotic resistance. Transl Res. 2017 Mar 1;181:59–70.

32. Kramer H, Kuffel G, Thomas-White K, Wolfe AJ, Vellanki K, Leehey DJ, et al. Diversity of the midstream urine microbiome in adults with chronic kidney disease. Int Urol Nephrol. 2018 Jun 1;50(6):1123–30.

33. Li X, Tang Y, Xu Z, Lin H, Wei S, Sheng J, et al. Impact of coexisting type 2 diabetes mellitus on the urinary microbiota of kidney stone patients. PeerJ. 2024 Feb 26;12:e16920.

34. Chen J, Zhao J, Cao Y, Zhang G, Chen Y, Zhong J, et al. Relationship between alterations of urinary microbiota and cultured negative lower urinary tract symptoms in female type 2 diabetes patients. BMC Urol. 2019 Aug 22;19(1).

35. Penckofer S, Limeira R, Joyce C, Grzesiak M, Thomas-White K, Wolfe AJ. Characteristics of the microbiota in the urine of women with type 2 diabetes. J Diabetes Complications. 2020 Jun 1;34(6).

36. Hrbacek J, Tlaskal V, Cermak P, Hanacek V, Zachoval R. Bladder Microbiota Are Associated with Clinical Conditions That Extend beyond the Urinary Tract. Microorganisms. 2022 May 1;10(5).

37. Li Y, Gao F, Chen J, Fan X, Zhan W, Wu P, et al. Characteristics of urinary microflora in women with type 2 diabetic peripheral neuropathy without lower urinary tract symptoms; [无下尿路症状的女性2型糖尿病周围神经病变患者的尿液菌群特征]. Chin J Urol. 2020;

38. Liu F, Ling Z, Xiao Y, Yang Q, Zheng L, Jiang P, et al. Characterization of the urinary microbiota of elderly women and the effects of type 2 diabetes and urinary tract infections on the microbiota [Internet]. Vol. 8, Oncotarget. 2017 p. 100678–90. Available from: www.impactjournals.com/oncotarget/

39. Düzgün Ç, Dede S, Karakuş E, Adaş M, Bilen Ö. Biochemical analysis of microbiotas obtained from healthy, prediabetic, type 2 diabetes, and obese individuals. Turk J Biochem. 2022;

40. Lin HJ, Chuang HN, Jhan PP, Ye HY, Lee IT, Hsiao TH, et al. Pyuria Is Associated with Dysbiosis of the Urinary Microbiota in Type 2 Diabetes Patients Receiving Sodium–Glucose Cotransporter 2 Inhibitors. Microbiol Res. 2023 Mar 1;14(1):34–41.

41. Calvigioni M, Biancalana E, Mazzantini D, Celandroni F, Rossi C, Mengozzi A, et al. In-depth Microbiological Characterization of Urine From Subjects With Type 2 Diabetes. J Clin Endocrinol Metab. 2024 Jun 13;dgae389.

42. Kim K, Lee S, Park SC, Kim NE, Shin C, Lee SK, et al. Role of an unclassified Lachnospiraceae in the pathogenesis of type 2 diabetes: a longitudinal study of the urine microbiome and metabolites. Exp Mol Med. 2022 Aug 1;54(8):1125–32.

43. Patel NB, Obregón-Tito AJ, Tito RY, Trujillo-Villaroel O, Marin-Reyes L, Troncoso-Corzo L, et al. Citroniella saccharovorans gen. nov. sp. nov., a member of the family Peptoniphilaceae isolated from a human fecal sample from a coastal traditional community member. Int J Syst Evol Microbiol. 2019 Apr 1;69(4):1142–8.

459 44. Zheng N, Guo R, Wang J, Zhou W, Ling Z. Contribution of Lactobacillus iners to Vaginal Health
460 and Diseases: A Systematic Review. Front Cell Infect Microbiol. 2021 Nov 22;11:792787.

461 45. Maillet F, Passeron A, Podglajen I, Ranque B, Pouchot J. Lactobacillus delbrueckii urinary tract
462 infection in a male patient. Médecine Mal Infect. 2019 May;49(3):226–8.

463 46. Hori E, Nakamura M, Tamura H. A diagnostically challenging case of pyelonephritis caused by
464 *Lactobacillus*. Clin Case Rep. 2022 Jun;10(6):e5999.

465 47. Pearce MM, Zilliox MJ, Rosenfeld AB, Thomas-White KJ, Richter HE, Nager CW, et al. The female
466 urinary microbiome in urgency urinary incontinence. Am J Obstet Gynecol. 2015
467 Sep;213(3):347.e1-347.e11.

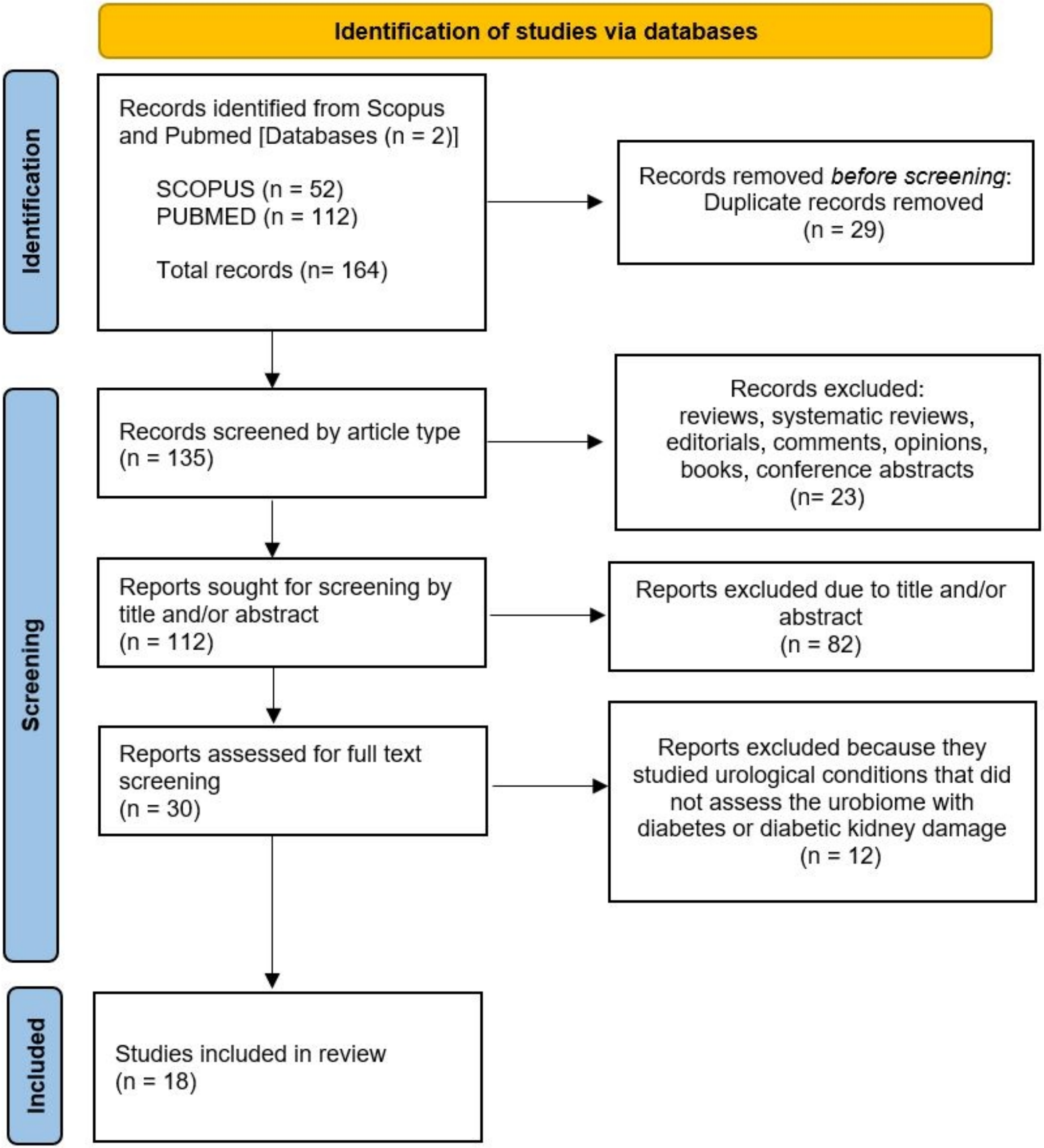
468 48. Kuo IC, Lee JJ, Hwang DY, Lim LM, Lin HYH, Hwang SJ, et al. Pyuria, urinary tract infection and
469 renal outcome in patients with chronic kidney disease stage 3–5. Sci Rep. 2020 Nov
470 10;10(1):19460.

471 49. Zaidi SMJ, Kaneez M, Almas T, Fatima L, Safian HA, Jamal AM, et al. Gauging the Risk Factors for
472 Asymptomatic Bacteriuria in Type-2 Diabetic Women: A Case-Control Study. Cureus [Internet].
473 2020 Jul 8 [cited 2024 Mar 5]; Available from: https://www.cureus.com/articles/35887-gauging-
474 the-risk-factors-for-asymptomatic-bacteriuria-in-type-2-diabetic-women-a-case-control-study

475 50. Yoo J, Groer M, Dutra S, Sarkar A, McSkimming D. Gut Microbiota and Immune System
476 Interactions. Microorganisms. 2020 Oct 15;8(10):1587.

477

478


Identification of studies via databases
Identification
Records identified from Scopus and Pubmed [Databases (n = 2)]
SCOPUS (n = 52)
PUBMED (n = 112)
Total records (n= 164)
Records removed *before screening*: Duplicate records removed (n = 29)
Screening
Records screened by article type (n = 135)
Records excluded: reviews, systematic reviews, editorials, comments, opinions, books, conference abstracts (n= 23)
Reports sought for screening by title and/or abstract (n = 112)
Reports excluded due to title and/or abstract (n = 82)
Reports assessed for full text screening (n = 30)
Reports excluded because they studied urological conditions that did not assess the urobiome with diabetes or diabetic kidney damage (n = 12)
Included
Studies included in review (n = 18)

Figure