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 may be 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 disease. 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
39 and diseases (2–4). The intricate structure of these microbial ecosystems within their ecological
40 niche, 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
44 lack understanding (4). Dysbiosis is a state where the microbiome composition or diversity are
45 disrupted or altered, or both, impeding normal functionality or causing low-grade inflammation that
46 may play a role in the onset and/or development of several diseases (5). Among the human
47 microbiomes, the gut microbiome is the best studied as it is seen as a "hidden" metabolic organ for
48 human wellbeing affecting immunity, neural, endocrinal or metabolic pathways (4,6). Gut dysbiosis
49 has been related to several diseases but if it is cause or effect of these diseases is a question that
50 remains unclear (4,7,8). While our understanding of the gut microbiome is evolving but limited, the
51 knowledge about other microbiomes is even more limited. This is particularly true for the urinary
52 tract microbiome, or urobiome, which was long believed to be sterile until molecular techniques
53 revealed its presence about a decade ago (9,10). The urobiome is characterized by a low biomass
54 relative to the gut microbiome, and its composition remains largely unexplored (9,11). Evidence
55 suggests that the urobiome differs between males and females. It is often associated with
56 lactobacilli in females, whereas in males is linked to *Corynebacterium* or *Staphylococcus* (9,11,12).
57 The differences in the urobiome between males and females have been attributed to the hormone
58 oestrogen, which plays a crucial role in glycogen production (11,13) and whose fluctuations with age
59 and menopausal status may affect the female urobiome (14). Bacteria of the genus *Lactobacillus*
60 metabolize glycogen, and as a byproduct of it there is lactic acid which acidifies the environment

61 modulating the growth of other bacteria (11). The acidic environment is assumed significant for the
62 protective role that lactobacilli play in both the vaginal and urinary microbiomes of women (9,13).
63
64 Diabetes, a disease characterized by elevated levels of glucose in the blood, is a leading cause of
65 cardiovascular disease, blindness or kidney damage (15). Diabetes, especially type 2 diabetes
66 mellitus (T2DM), has become a global health emergency whose prevalence have been rising for
67 decades affecting 537 million people globally in 2021, including 6.7 million related deaths (15,16).
68 The incidence of diagnosed diabetes in adults is stabilising in some high-income countries, but
69 incidence in children is still increasing (17–20). Impaired glycaemia contributes to enhanced risk of
70 infections, and it may explain why diabetic individuals have higher risk of urinary infections,
71 pyelonephritis and urosepsis (21). Not only individuals with diabetes have higher glucose, albumin
72 and other proteins in urine which may affect the urobiome composition, enhance bacterial growth
73 of some species and may influence the diversity of the urobiome; but also they have high glucose in
74 urine which compromises the immune response and uroepithelial integrity (21). However, we have
75 limited information about the composition of the urobiome related to diabetes. In addition, damage
76 in the kidney blood vessels, because of diabetes or other causes, may decline the estimated
77 glomerular filtration rate (eGFR) which impacts urinary functions as these organs are connected
78 through the ureters to the urinary bladder (22). Development of diabetic nephropathy may further
79 turn in chronic kidney disease (CKD) or end stage renal disease (ESRD), the former associated with
80 diabetes but also other comorbidities while the latter is mostly caused by diabetes (22).
81 As there is a clear knowledge gap regarding the role of the urobiome in diabetes and diabetic kidney
82 disease (DKD), the aim of this systematic review is to gather available data and synthetise the main
83 findings about the urobiome, particularly, the urinary microbiota in association with diabetes and
84 kidney conditions derived from diabetes such as CKD or ESRD.
85

86 **2. Materials and methods**

87 2.1 Search strategy

88 The comprehensive literature search was conducted according to the Preferred Reporting Items for
89 Systematic Reviews and Meta-analyses (PRISMA) guidelines (23). It was registered under accession
90 number of 565545 in the International Prospective Register of Systematic Reviews (PROSPERO)
91 hosted by the National Institute for Health and Care Research of the University of York (United
92 Kingdom). A comprehensive literature search on SCOPUS and PUBMED databases was conducted
93 until July 2024. We used the 'TITLE-ABS-KEY' search in SCOPUS (Web of Science) database while 'ALL
94 FIELDS' were used in PUBMED database. The search included the following terms [("urobiome" OR
95 "urinary microbiome" OR "urinary microbiota" OR "urinary tract microb*" OR "urine microb*" OR
96 "urogenital microbiome" OR "urogenital microbiota") AND ("kidney disease*" OR "chronic kidney
97 disease" OR "diabetes" OR "albuminuria" OR "diabetic nephropathy" OR "end stage renal disease*"
98 OR "renal disease*")] and it wanted to identify relevant clinical urinary microbiome studies
99 associated with diabetes and/or kidney impairment published from 1st January 2000 until 4th July
100 2024.

101 2.2 Study selection

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

111 2.3 Data extraction, assessment of quality and risk of bias

112 Throughout this synthesis, we used the PRISMA and PROSPERO frameworks and we incorporated
113 the associated guidance at each stage. Following the removal of duplicates, the first step was the
114 screening of studies by article type. After removal of non-suitable studies, the second step included
115 a screening of studies by title and/or abstract for relevance. Those that seemed relevant were the
116 preliminary selected publications and they were finally screened based on full-text information.
117 Studies lacking full-text availability or with missing or unclear information were excluded from the
118 evaluation (**Fig. 1**). Two independent authors (T.G and Y.T.L) screened studies by title and abstract
119 and, if discrepancies arose, they were discussed until they reached a common agreement.
120 Preliminary selected publications were fully screened by T.G., reviewed by Y.T.L, and if they fulfilled
121 the inclusion criteria, they were selected for this systematic review. Studies quality and risk of bias
122 were assessed on participant selection, exclusion criteria, data measurements and analysis (25,26). A
123 determination of "low risk" or "high risk" bias according to the Cochrane and the National Heart,
124 Lung and Blood of the United States (NHLBI) guidelines was assessed by T.G. and independently
125 checked by Y.T.L and J.Ä. (25,27). Any disagreement was discussed and resolved between these
126 authors.

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

129

130 **3. Results**

131 In total, eighteen studies were included including cross-sectional case-control studies, cross-
132 sectional surveys and one prospective longitudinal study (**Table 1** and **Supplementary Table 1**).
133 These studies altogether sequenced the urinary microbiome of 1,571 people, some participants
134 were part of the same cohort studied and were included multiple times (28–31). Of these individuals
135 662 had diabetes, 609 were healthy individuals, 179 had prediabetes or were at risk of diabetes, and
136 121 did not have diabetes but had other comorbidities. Among the 662 diabetic individuals, 36

137 individuals had diabetic kidney disease (32–34) while one studied CKD patients, most of them with
138 diabetes, but did not mention the primary cause of CKD (35). Sixteen of the studies used voided
139 midstream urine samples, or a modification of this collection technique, to avoid as much as possible
140 skin or genitalia microorganisms (**Supplementary Table 1**). The remaining two studies analysed
141 urinary bladder microbiota (catheterised urine) and, among these two, one was focused only on
142 males (**Table 1**). Eight studies included only female samples (four of these used the same cohort
143 data); and nine studies had mixed female-male data (**Table 1**). All studies except one used 16S
144 sequencing which included twelve studies with small sample sizes with less than 100 individuals,
145 including five studies with less than 50 participants (**Table 1**). The only one that used shotgun
146 sequencing had a small sample size (n = 29) (**Supplementary Table 1**). Participants of these 18
147 studies were adults; individuals with diabetes ranged between 50 and 72 years old on average, and
148 healthy controls were similar in age but slightly younger (if age was significantly different between
149 groups, it is stated in the comments in **Table 1**). Selected studies had comprehensive exclusion
150 criteria which are detailed in **Supplementary Table 1** and they were assessed for risk of bias in
151 **Supplementary Table 2**. Moreover, as articles considered confounding factors, such as age, BMI or
152 other relevant clinical factors; those that were statistically significant between groups are pointed up
153 in the comments of **Table 1.**

154 Among the 11 case-control studies, 9 reported richness and α-diversity analyses of which three of
155 the studies observed a decrease in richness and α-diversity, one a decrease in richness, two a
156 decrease in α-diversity, one observed an increase in α-diversity but this study assessed kidney stones
157 patients with and without diabetes, and two studies did not observe differences in richness or α-
158 diversity in the urobiome of the individuals with diabetes compared to those without diabetes
159 (**Table 1**). Among the studies assessing only diabetic individuals, one study observed lower α-
160 diversity in diabetic individuals with pyuria, one study observed lower richness in patients with
161 diabetic peripheral neuropathy, one study did not observed differences in α-diversity when
162 assessing urinary interleukin-8; and one study observed increased richness in diabetic individuals

163 compared to diabetic individuals with hyperlipidaemia but decreased richness if compared to
164 diabetic individuals with hypertension. Lastly, the two studies assessing CKD reported a decrease of
165 α-diversity as CKD worsened independently of the primary cause of CDK (diabetes or other). Indexes
166 calculated in each study are detailed in **Supplementary Table 3** and the main ones are detailed in
167 **Table 1.** Only seven studies reported β-diversity analyses with variable results (**Table 1**); 4 reported
168 differences in β-diversity, of which 2 described different urotypes (35–38); while 3 did not see
169 differences in β-diversity(29,39,40).

170 Significant results in microbial species for diabetic adults (statistical significance set to $p < 0.05$) of
171 each article are summarised in **Table 1** and all details can be found in **Supplementary Table 4**.
172 Comprising all studies, most of the bacterial species were identified at the genus level; few at species
173 level and some at higher taxonomy levels. The relative abundance of 69 different bacteria was
174 decreased, of 53 was increased in the urine of diabetic individuals while bacteria such as
175 *Enterococcus, Stenotrophomonas, Klebsiella* or Actinobacteriota had increased or decreased relative
176 abundance depending on studies (**Supplementary Table 4, Supplementary Table 5** and **Table 1**).

177 Overall, we could observe that the genus *Lactobacillus,* and at species level *Lactobacillus iners*, had
178 increased relative abundance in diabetic individuals in 6 and 2 studies, respectively; and it was
179 related to poor glycaemic control and with presence of interleukin-8 in urine (29,30,36–38,41).
180 Three studies reported a decrease in relative abundance of the family *Bifidobacteriaceae* in urine of
181 individuals with diabetes which includes the genera *Bifidobacterium* and *Gardnerella;* while one
182 study reported the absence of the genus *Gardnerella* in individuals with diabetes and hyperlipidemia
183 (note: *Gardnerella* is included now in the genus *Bifidobacterium*)(29,30,32,42). In addition, depletion
184 of the family *Peptoniphilaceae* in urine of T2DM patients was reported in several selected studies. In
185 fact, studies reported decreased relative abundance of this family being relevant genera
186 *Peptoniphilus*, *Anaerococcus, Finegoldia, Murdochiella* and *Gallicola*, which were reported decreased
187 abundant in four, three, two, one and one article, respectively (28,29,37,38). The genus *Parvimonas,*

188 which belongs to this family as well, was increased in relative abundance in diabetic individuals
189 compared to non-diabetics only in the elderly cohort (41); while its relative abundance was
190 increased in the diabetic cohort compared to the diabetic plus hypertension cohort (30). Lastly,
191 three studies reported increased relative abundance of *Escherichia* (*Escherichia-Shigella),* a well-
192 known genus causing urinary tract infections (UTI), which was related to poor glycaemic control and
193 in two studies this genus was related to urinary symptoms or pyuria in urine (even though
194 individuals did not have an active UTI) (34,37,43).

195 Taxonomic classification of bacteria identified in selected studies was updated according to Genome
196 Taxonomy Database release 220 (https://gtdb.ecogenomic.org/) at higher taxonomical levels (e.g.
197 phylum) but at lower levels was maintained to preserve clarity of the conclusions. For the most
198 accurate taxonomic annotation refer to details in **Supplementary Table 5**.

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 (28); [£] = 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								
(36)	China	30	0 vs 15	Kidney stones and T2DM: 56.20 ± 11.41 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) ↑ α- diversity (Shannon index) β-diversity: the group with kidney stones alone and with kidney stones and T2DM clustered and could be differentiated	↑*Sphingomonas* ↑*Propionibacterium* ↑ *Corynebacterium* ↑ *Cellulosimicrobium* ↑ *Methylophilus* ↑ *Lactobacillus* ↑ *Enhydrobacter* ↑ *Chryseobacterium* ↑ *Haemophilus* ↑ *Allobaculum*	The relative abundance of *Enhydrobacter, Chryseobacterium,* and *Allobaculum* genera exhibited correlations with fasting blood glucose and HbA1c values.
(44)	Italy	75	25 vs 50	H males: 62 ± 7 H females: 57 ± 6 T2DM males: 65 ± 9 T2DM females: 64 ± 10		No differences in richness or α-diversity (observed species, Chao, and Shannon indexes)	NS [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.

(33)	China	34	15 vs 19	H: 43.73 ± 7.54 T2DM: 59.95 ± 9.38	T2DM with diabetic kidney disease	↓ α - diversity in T2DM (Shannon and Simpson indexes)	↓Bacillota ↓ Bacteroidota ↑ Pseudomonadota ↑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*.
(45) [£]	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.
(39) [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) ↓ α - diversity (Shannon and Simpson indexes) 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. Smokers had higher α - diversity. No significant differences in α - diversity for hypertension or CKD. β – diversity was different between individuals with (1) CKD vs no CKD; (2) severe vs mild urinary symptoms; and (3) antibiotic prophylaxis vs without
(42) [¥]	Turkey	60	15 vs 15	H: 41.93 ± 11.3 Obese: 41.86 ± 8.53 Pre-DM: 49.6 ± 8.91 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
(38)	United States	136	49 vs 87	H: 50.5 ±10.6 T2DM: 51.4 ± 10.9	Females with T2DM	β – diversity: Both H and T2DM clustered in 4-5 urotypes Urotypes in T2DM:	↑ *Lactobacillus* ↓ *Corynebacterium* ↓ *Staphylococcus* [T2DM differences in presence/absence:	H and T2DM groups differed significantly in FBG and HbA1c (both ↓in H). HbA1c was positively correlated with *Lactobacillus* and negatively associated with *Prevotella* and *Corynebacterium* in all cohort; and

						Lactobacillus and *Enterobacteriaceae* vs H females: *Gardnerella* and mixed	*Lactobacillus* was more present while *Corynebacterium, Anaerococcus, Finegoldia* and *Peptoniphilus* were less present]	negatively associated with *Prevotella* also in the T2DM group. Prevalence of *Lactobacillus* increased with weight; and most of participants were obese.
(37)	China	58	26 vs 32	H: 57.62 ± 9.24 T2DM: 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) β - diversity was different between H and T2DM	↑ *Escherichia-Shigella* ↑ *Klebsiella* ↑ *Aerococcus* ↑*Delftia* ↑*Enterococcus* ↑*Alistipes* ↑*Stenotrophomonas* ↑ *Micrococcus* ↑ *Deinococcus* ↓ *Gallicola* ↓*Arcobacter* ↓*Arcanobacterium* ↓*Kocuria* ↓ *Murdochiella* ↓ *Solitalea* ↓ *Peptoniphilus*	FBG was ↑ in T2DM compared to H group. HbA1c was ↑ in T2DM with urinary symptoms vs T2DM without. The diabetic group with urinary symptoms (compared to the group without) had lower richness, different β – diversity; and: ↑*Escherichia-Shigella* ↑ *Campylobacter* ↑ *Megasphera* ↓ *Prevotella* ↓ *Dialister* ↓ *Anaerococcus* ↓ *Fusobacterium* ↓ *Prevotella_6,* ↓ *Mycoplasma* ↓ *Peptoniphilus* ↓ *Porphyromonas* ↓ *Fodinicola* ↓ *Brevundimonas* The diabetic group with high HbA1c compared to the diabetic low HbA1c group had lower α – diversity, different β – diversity, and: ↑*Escherichia-Shigella* ↑ *Lactobacillus* ↓ *Prevotella* ↓ *Campylobacter* ↓ *Dialister* ↓ *Anaerococcus* ↓ *Peptoniphilus* ↓ *Porphyromonas* ↓ *Fodinicola*
(41)	China	100	50 vs 50	Elderly: 71.86 ± 6.70 Non-old: 50.06 ± 7.51	Elderly vs non-elderly cohorts. Half of the females of each cohort had T2DM	↓ richness (OTU and Chao indexes) in elderly with T2DM No differences in α - diversity (Shannon and Simpson indexes) between the	Elderly with T2DM (compared to elderly without): ↓ *Nitrospirae* ↓ *Odoribacter* ↓*Aeromonas*	T2DM females treated with metformin. *Lactobacillus* had a decreased trend in T2DM patients with FBG > 10 mmol/l. *Lactobacillus iners* was not associated

						elderly with and without T2DM	↓*Clostridium* ↓*Enterobacter* ↓*Butyricimonas* ↓*Enterococcus* ↓ *Erwinia* ↓ *Fusobacterium* ↓ *Klebsiella* ↓ *Lachnobacterium* ↓*Stenotrophomonas* ↓*Phascolarctobacterium* ↑*Eggerthella* ↑ *Parvimonas* ↑*Bdellovibrio* ↑*Hydrogenophaga* ↑***Lactobacillus iners***	with FBG. 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.*
(28)	China	140	70 vs 70	Matched H and T2DM individuals. 70% > 56 years or older; only 8% younger than 45 years	Authors matched individual characteristics between the H and the T2DM groups	↓ richness (number of OTUs, observed species, ACE and Chao1 indexes) ↓ α-diversity (Shannon index)	↑ Actinobacteria ↑ *Porphyromonas* ↑ *Flavobacteriales* ↑ *Collinsella* ↓ *Akkermansia muciniphila* ↓ Bacteroidota ↓ *Prevotellaceae* ↓ *Prevotella* ↓*Pseudomonadales* ↓*Peptoniphilus* ↓ *Citrobacter* ↓Actinobacteriota ↓ *Synergistota* ↓*Acinetobacter* ↓*Campylobacter* ↓*Campylobacterales* ↓ *Staphylococcus* ↓ *Anaerococcus* ↓ *Halomonas* ↓ *Finegoldia* ↓ *Streptococcus* ↓*Veillonella* ↓*Clostridium* ↓*Corynebacterium*	Actinobacteria increase was associated with higher BMI; increased FBG and urine glucose. *Akkermansia muciniphila* decrease was associated with FBG and urine glucose. Urine glucose, FBG, urine infections, hypertension and hyperlipidemia were statistically ↑ in T2DM compared to H group. Authors studied hypertension and hyperlipidemia in article by (30); inflammatory markers in article by (Ling et al. 2017) and the effect on diet in article 7 (31).
(34) Ω	United States	29	8 vs 6	H: 34.57 ESDR T1DM: 51.00 ESDR T2DM: 64.33 (all ESRD: 53.28)	Transplant ESRD patients. Kidney damage caused by 4 primary diagnoses: other, T1DM, T2DM, and hypertension. 15 individuals had	↓ α-diversity (Shannon and inverse Simpson indexes) ↓ richness (observed species) β-diversity: primary diagnoses had no significant differences in bacterial	↑ *Enterococcus faecalis* ↑ *Enterococcus faecium* ↑ *Enterococcus sp.* ↑ *Escherichia coli* ↑ *Escherichia sp.* ↓ *Cutibacterium acnes* ↓ *Corynebacterium*	↑ Bacillota and ↓Actinobacteria because *Enterococcus* belongs to the former phylum while as *Mobiluncus* belongs to the latter. Both groups had similar folate metabolism (which is the target of trimethoprim - sulfamethoxazole) but

						non-diabetic ESRD. Trimethoprim - sulfamethoxazole was given prophylactically	composition; but all had with the H	↓*Mobiluncus curtisii*	had differences in specific enzymes. Dihydrofolate synthase/folypolygluta-mate synthase was increased in transplant groups.
Studies with diabetic individuals only									
(43)		Taiwan	7	0 vs 7	T2DM No-pyuria: 59 ± 5.7 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
(40)		China	30	0 vs 30	T2DM with diabetic peripheral neuropathy: 55.82 ± 7.83 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) α – diversity was no different (Shannon and Simpson indexes) β – diversity was no different	Group with DPN: ↑ *Mycoplasmataceae* ↓Propionibacteriaceae ↓ Pseudobdellovibrioinaceae ↓Sphingobacteriaceae	T2DM females: 17 with DPN and 13 without. Both groups did not differ in clinical characteristics.
*	(30)	China	70	0 vs 70	T2DM: 56.28 ± 13.91 T2DM + hypertension: 70.42 ± 9.00 T2DM + hyperlipidemia: 54.43 ± 10.66 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) ↓ richness compared to hypertension groups (number of reads and number of OTUs) 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> ↑ *Lactobacillus iners* ↑ *Acinetobacter rhizosphaerae* ↑ *Acinetobacter schindleri* ↑*Lactobacillus* ↑*Enterobacter* ↑*Klebsiella* ↑*Shuttleworthia* ↑*Sneathia* ↑ *Parvimonas* ↑*Megasphera* ↑*Erwinia* ↓ *Kocuria palustris* ↓ *Aeromonas* ↓ *Roseburia* ↓ *Ruminococcus* <u>Compared to T2DM + hyperlipidemia, the group **with only** T2DM had:</u> ↓ *Faecalibacterium* ↓ *Collinsella* ↓ *Oscillospira* (diagnostic factor to differentiate both groups)	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. 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). 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 + hyperlipidemia *Prevotella, Lactobacillus* and *Bacillus*.

								<u>Compared to T2DM + hypertension and hyperlipidemia, the group **with only** T2DM had:</u> ↑*Acinetobacter rhizosphaerae* ↑ *Shuttleworthia* ↓*Gemella* ↓*Prevotella* <u>Compared to T2DM + hypertension and hyperlipidemia, the group with T2DM + hyperlipidemia had:</u> ↑*Acinetobacter rhizosphaerae* ↑*Faecalibacterium* <u>Compared to T2DM + hypertension, the group with T2DM + hyperlipidemia had:</u> ↑ *Klebsiella* ↑ *Novosphingobium* ↑ *Lactobacillus* ↑ *Enterobacter* <u>The two groups with hypertension did not show differences</u>	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. 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.
*	(29)	China	70	0 vs 70	T2DM with IL-8 in urine: 65.78 ± 13.58 T2DM without IL-8 in urine: 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) β-diversity: the group with Il-8 and without IL-8 in urine clustered together and could be differentiated	T2DM females without IL-8: ↓*Bifidobacteriaceae* ↓*Shuttleworthia* ↓ *Thermales* ↓ *Streptococcus anginosus* ↓ *Acinetobacter rhizosphaerae* ↓ *Acinetobacter schindleri* ↓ *Lactobacillus iners* ↓ *Akkermansia muciniphila* ↓ *Mobiluncus* ↓ *Peptoniphilus* ↓ *Corynebacterium* ↓ *Gemella* ↓ *Enterococcus* ↓ *Geobacillus* ↑ *Prevotella copri* ↑ *Prevotella stercorea* ↑*Bacteroides uniformis* ↑*Coprococcus eutactus* ↑ *Faecalibacterium*	*Ruminococcus* was positively associated with urinary IL-8. Urinary IL-8 was high in T2DM patients with ↑ *Pseudomonas* and ↑ *Klebsiella*. 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)

								↑ *Megamonas* ↑ *Comamonas* ↑*Pseudomonas* ↑*Phascolarctobacterium*	
*	(31)	China	140	70 vs 70	Matched H and T2DM individuals. 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.
Case-control studies with individuals that have kidney or renal disease									
(32)		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* ↓ *Dermabacter* ↓*Gardnerella*	Apart from diabetes in CKD patients, significance was found for sex: *Lactobacillus* linked to females; *Staphylococcus* and *Anaerococcus* to males.
(35)		United States	77	23 vs 54	Males: 76.6 ± 7.6 Females: 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. Urinary incontinence and ↑ eGFR was associated with ↑ α-diversity.

204 **4. Discussion**

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

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

230 acids such as butyrate (*Peptoniphilus, Anaerococcus,* and *Gallicola*) and acetate (*Peptoniphilus,*
231 *Parvimonnas, Gallicola* and *Finegoldia)* or lactate *(Murdochiella)* (46). *Parvimonas* increased in
232 relative abundance in diabetic individuals compared to non-diabetics only in the elderly cohort (41);
233 while its relative abundance was increased in the diabetic cohort compared to the diabetic plus
234 hypertension cohort (30). In the latter, if diabetes is accompanied by other factors such as
235 hypertension, there is decreased abundance of this genus pointing to a depletion as the health
236 status worsens. *Peptoniphilus* and *Anaerococcus* abundance may be associated to glycosylated
237 haemoglobin HbA1c as diabetic individuals with high HbA1c had decreased relative abundance of
238 these two genera in urine compared to diabetic individuals with low HbA1c (37).

239 The genus *Lactobacillus* seems to be more abundant in the urine of diabetic individuals according to
240 this systematic review. *Lactobacillus iners*, which has been described as a transitional species that
241 colonizes the vagina after an ecological disturbance (47), was found enriched in the urine of T2DM
242 females (30,41) and in the same cohort with higher levels of inflammatory interleukin-8 in urine (29).
243 Other species of this genus such as *Lactobacillus crispatus* and *Lactobacillus gasseri* have been
244 associated with lack of lower urinary tract symptoms and lack of urgency urinary incontinence,
245 respectively; and levels of *Lactobacillus* in the vagina are reported to be associated with menopausal
246 status and with reduced risk of UTI (38). Although lactobacilli have generally been considered
247 beneficial and the normal microbiota of the urobiome and vagina, there is increasing evidence that
248 some *Lactobacillus* spp. may be pathogenic or related to urinary symptoms (48,49). The association
249 between the different species of *Lactobacillus* and their role in T2DM needs further investigation,
250 considering that age, oestrogen, HbA1c and/or BMI could also be important factors affecting the
251 abundance of this genus. In this context, HbA1c was positively correlated with *Lactobacillus.*
252 Individuals with T2DM and high HbA1c had higher abundance of this genus compared to those with
253 T2DM and low HbA1c levels (37,38). Another point to consider is that the genus *Lactobacillus* is
254 naturally resistant to widely used antibiotics and T2DM individuals often have multiple infections

255 that require the use of them (37). Thus, we could hypothesize that antibiotic therapy may imbalance
256 the urobiome allowing *Lactobacillus* to grow excessively which may induce inflammation.

257 Diabetic, prediabetic and obese individuals had lower abundance of the genus *Bifidobacterium* in
258 urine; and the abundance of this genus was negatively correlated with age (41,42). In this line, T2DM
259 patients with hyperlipidemia lacked *Bifidobacterium* (former *Gardnerella*) (30). This was also true for
260 the former genus *Gardnerella,* that is now included in the genus *Bifidobacterium,* that have
261 decreased abundance in CKD patients with diabetes (32). *Bifidobacterium* (*Gardnerella*) was a
262 common urotype in healthy females compared to diabetic females that had *Lactobacillus* or
263 *Enterobacteriaceae* urotypes (38). *Bifidobacteriaceae* had decreased relative abundance in diabetic
264 females without interleukin-8 in urine in the study by Ling et al; but this group had also lower age
265 and BMI compared to the group with interleukin-8 (29). These results may imply that diabetes but
266 also age, BMI or levels of interleukin-8 could influence the relative abundance of *Bifidobacterium* in
267 urine (29).

268 The role of the genus *Prevotella* in the urinary microbiota is unclear. Although HbA1c was negatively
269 correlated with *Prevotella* in T2DM patients (38) and Chen et al. reported a decrease of this genus in
270 individuals with diabetes with high urinary symptoms (37), other studies have found that this genus
271 was more prevalent in T2DM (44) or that urotypes and enrichment of *Prevotella* was associated with
272 urinary incontinence patients (12,50). Therefore, similarly to findings with the genus *Lactobacillus,*
273 we will likely need higher resolution studies, preferably shotgun metagenomics, to identify which
274 species or strains of these genera may play different roles in the urobiome.

275 UTIs are common in diabetic patients and are associated with poor glycaemic control, which
276 compromises kidney function and impairs quality of life (51). That is why many selected articles also
277 examined urinary symptoms, as hospitalization for UTIs caused by diabetes is more than twice as
278 common as hospitalizations for UTIs caused by other factors. The use of sodium–glucose
279 cotransporter 2 inhibitors as therapy for diabetes may contribute as these drugs reduce glucose

280 reabsorption, thus lowering the level on blood, but causing glycosuria in urine which could increase
281 the risk of UTIs in those patients (52). Hyperglycaemia and diabetes has been shown to reduce
282 psoriasin and occludins levels in the urinary tract leading to be more prone to urinary tract infections
283 (21). Moreover, psoriasin is an antimicrobial peptide with a broad protective role against *Escherichia*
284 *coli* and *Enterococcus* urinary infections, two of the most common pathogens causing UTI in
285 diabetics (21). In addition, low-grade inflammation with higher concentration of proinflammatory
286 cytokines and the depletion of producers of short-chained fatty acids such as butyrate, which has
287 beneficial effects on the host (53), may affect mucosa integrity, bacterial translocation and host–
288 urine microbiota interactions in T2DM patients.

289 The results of this systematic review have also highlighted that urinary microbiota diversity seems
290 lower in CKD or ESRD and it drops as the disease progresses independently of the primary cause
291 (32,35). Diabetes progression to kidney damage reduces eGFR and as eGFR declines the excretion of
292 uromodulin follows the same trend. Uromodulin, an urinary antimicrobial peptide whose function is
293 to stick to bacteria forming larger particles which enhance their excretion by the kidney, is produced
294 exclusively in the renal tubules and is affected by the decline of renal function (35) which may also
295 influence bacterial growth, microbiota diversity and risk of UTI.

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

301 <u>Strengths and limitations</u>

302 To our knowledge, this review represents the first comprehensive analysis of the current
303 understanding of the urobiome, specifically the urinary microbiota, in the context of diabetes. One
304 notable strength of this review is the identification of emerging trends within the urobiome across

305 multiple studies where the majority had low risk of bias assessment. For instance, this is the first
306 article to highlight the significance of the *Peptoniphilaceae* family within the urobiome of diabetic
307 individuals, which has frequently been reported as less prevalent or reduced in relative abundance
308 in urine of diabetic individuals. Additionally, this work is strengthened by its systematic approach,
309 adhering to PRISMA and PROSPERO guidelines, ensuring standardized and reproducible results.

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

324 **5. Future perspectives**

325 There are significant knowledge gaps regarding the role and impact of the urobiome in both health
326 and disease. This an emerging field that has attracted increasing attention in recent years, but the
327 influence of the urobiome on conditions such as diabetes and diabetic kidney disease remains
328 largely unexplored. While this systematic review has identified common trends across studies—such

329 as the depletion of *Peptoniphilaceae* and the increased abundance of *Lactobacillus* in the urine of
330 diabetic adults—the evidence remains insufficient due to the small sample sizes of most studies and
331 the limited resolution provided by 16S sequencing.

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

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353

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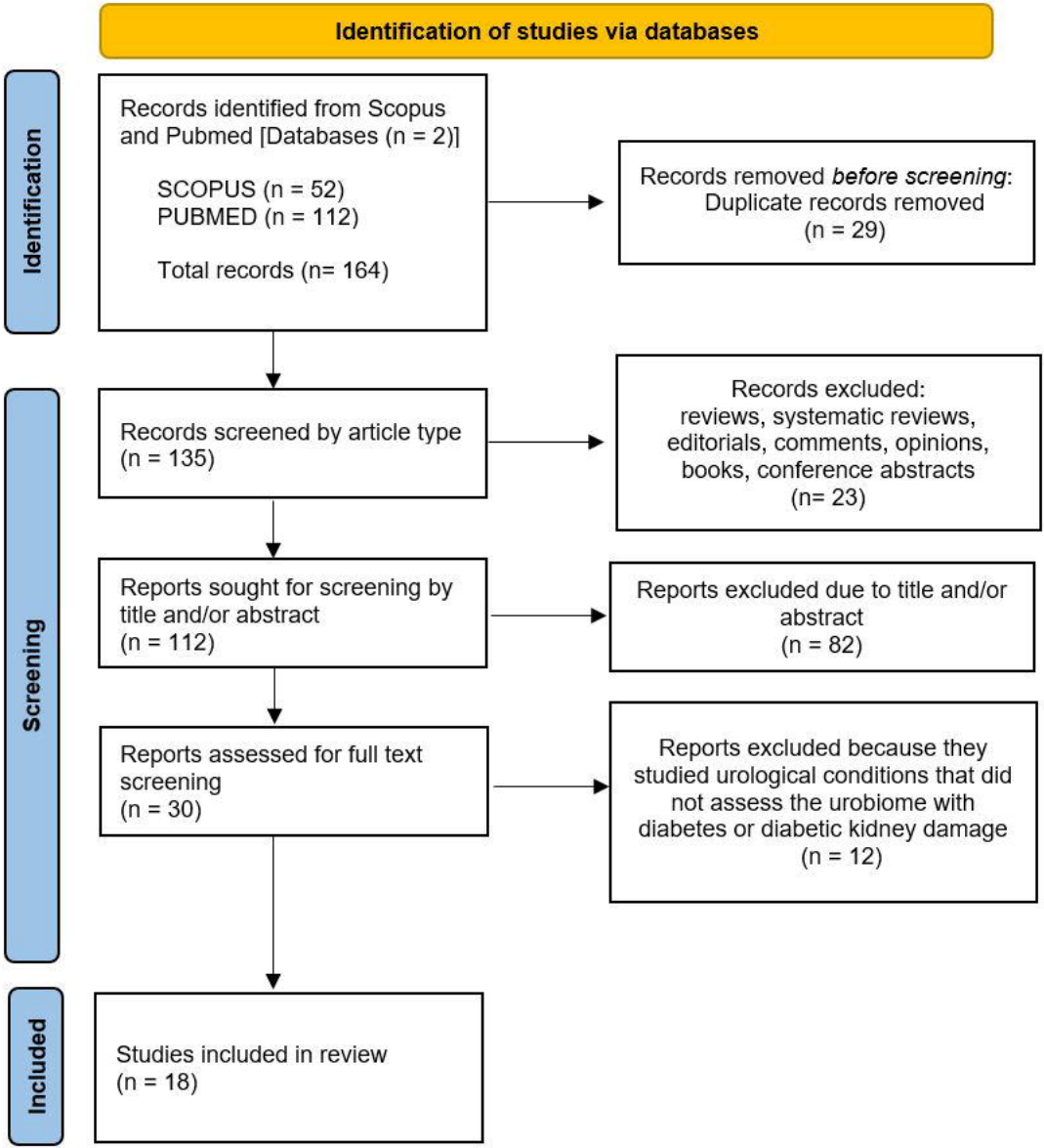
Identification of studies via databases
Identification
Records identified from Scopus and Pubmed [Databases (n = 2)]
SCOPUS (n = 52)
PUBMED (n = 112)
Total records (n= 164)
Records removed *before screening*: Duplicate records removed (n = 29)
Screening
Records screened by article type (n = 135)
Records 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)