The Potential Role of Urinary Microbiota in Bladder Carcinogenesis: A Systematic Review

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Purpose: The quantitative objective of the current systematic review was to identify the potential role of urinary microbiota in bladder cancer (BC) carcinogenesis, invasiveness, progression, and metastasis.

Materials and Methods: The proposed systematic review was conducted in accordance with critical review according to the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) statement, and the Joanna Briggs Institute (JBI) methodology for systematic reviews. The search strategy aimed to find both published and unpublished studies up to the January 2024. A JBI appraisal checklist was used to assess possible biases.

Results: This systematic review was centered on 27 studies comprising 926 BC patients. Overall, 412 control individuals were compared with BC patients. The most common sampling method was midstream urine collection. Regarding microbial alpha diversity, there was no statistically significant difference between cancerous and healthy samples (n = 8), recurrent and not recurrent (n = 1), responders versus non-responders(n = 1), tumor grades (n = 1), and collection methods (n = 1). However, five studies reported higher diversity in controls, and five other studies reported, conversely, high levels of alpha diversity in BC patients or recurrent cases. Furthermore, a responder (RE) to treatment and a non-muscle invasive bladder cancer (NMIBC) groups demonstrated significant difference with non-responder (NR) and muscle invasive bladder cancer (MIBC), respectively. In terms of beta-diversity, nine studies reported significant diversity between BC patients and controls, one article demonstrated difference between recurrent and not recurrent patients, a study reported significant difference in RE and NR groups whereas another showed opposite, and others (n = 4) did not find any difference between BC, controls, MIBC and NMIBC patients, or between tumor grades. One study reported a difference between the collection method and beta-diversity in males and another reported the difference in females.

Conclusion: The included studies demonstrate that the composition of urinary microbiota is altered in patients with BC. However, the differentially enriched genera in the urine of these patients vary between studies, and there is too much heterogeneity across studies to make any reliable and valid conclusions. Furthermore, well-designed research is necessary to assess the role of microbiota in the carcinogenesis and progression of BC.

Keywords: Microbiota; Microbiome; Urinary Bladder Neoplasms; Systematic Review.

INTRODUCTION

Recent advances in molecular analysis and non-culture-based technologies has allowed researchers to identify the diverse number of microorganisms residing in our body environment called microbiota(1). Microbiomes are individual to each organism; in tune with the microbial profile of other organs, intra-individual microbial variability has been elucidated in the urinary tract(2-4). It is also well-known that the urinary tract contains an overall low microbial biomass. Despite tremen-

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Received November 2023 & Accepted May 2024

Table 1. Quality assessment of the included studies based on Joanna Briggs Institute critical appraisal checklists (https://jbi.global/critical-appraisal-tools)

Study ID	Year	Study Design	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10	Overall quality
Bi et al.	2019	Cross-sectional	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes			80%< good
Mansour et al.	2020	Cross-sectional	Unclear	Yes	Yes	Yes	No	No	Yes	Yes			60%-80% medium
Hourigan et al.	2020	Cross-sectional	Yes	Yes	Yes	Yes	No	No	Yes	Yes			60%-80% medium
Chipollini et al.	2020	Cross-sectional	No	Yes	Yes	Yes	No	No	Yes	Yes			60%-80% medium
Zeng et al.	2020	Cross-sectional	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes			80%< good
Hussein et al.	2021	Cross-sectional	Yes	Yes	Yes	Yes	Unclear	Unclear	Yes	Yes			60%-80% medium
Pederzoli et al.	2020	Cross-sectional	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes			80%< good
Popovi□ et al.	2018	Cross-sectional	No	Yes	Yes	Yes	No	No	Yes	Yes			60%-80% medium
Wu et al.	2018	Cross-sectional	Unclear	Yes	Yes	Yes	Yes	Yes	Yes	Yes			80%< good
Oresta et al.	2021	Cross-sectional	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes			80%< good
Mansour et al.	2022	Case-control	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	NA	Yes	80%< good
Mai et al.	2019	Cross-sectional	Unclear	Yes	Yes	Yes	No	No	Yes	Yes			60%-80% medium
Qiu et al.	2022	Cross-sectional	Yes	Yes	Yes	Yes	No	No	Yes	Yes			60%-80% medium
Ma et al.	2021	Case-control	NA	NA	Yes	Yes	Yes	NA	NA	Yes	NA	Yes	80%< good
Liu et al.	2019	Cross-sectional	Yes	Yes	Yes	No	No	Yes	Yes	No			60%-80% medium
Qin et al.	2022	Cross-sectional	Yes	Yes	Yes	No	No	Yes	Yes	No			60%-80% medium
Parra-Grande et al.	2022	Cross-sectional	Yes	Yes	Yes	No	No	Yes	Yes	No			60%-80% medium
Sun et al.	2023	Cross-sectional	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes			80%< good
Zhang et al.	2023	Cross-sectional	Yes	Yes	Yes	No	No	Yes	Yes	No			60%-80% medium
Ozer et al.	2021	Cross-sectional	Yes	Yes	Yes	No	No	Yes	Yes	No			60%-80% medium
Hrbáček et al.	2023	Case-control	Unclear	NAa	Yes	Yes	Yes	Yes	Yes	Yes	NA	Yes	80%< good
James et al.	2023	Cross-sectional	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes			80%< good
Hussein et al.	2023	Cross-sectional	Yes	Unclear	Yes	Yes	Yes	Yes	Yes	Yes			80%< good
Pederzoli et al.	2023	Cross-sectional	Unclear	Yes	Yes	Yes	Unclear	Yes	Yes	Yes			60%-80% medium
Liu et al.	2024	Case-control	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	NA	Yes	80%< good
Nardelli et al.	2024	Cross-sectional	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes			80%< good
Heidrich et al.	2024	Cross-sectional	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes			80%< good

JBI critical appraisal checklist for Case-control studies:
Q1. Were the groups comparable other than the presence of disease in cases or the absence of disease in controls? Q2. Were cases and controls matched appropriately? Q 3. Were the same criteria used for identification of cases and controls? Q4. Was exposure measured in a standard, valid and reliable way? Q 5. Was exposure measured in the same way for cases and controls? Q6. Were confounding factors identified? Q 7. Were strategies to deal with confounding factors stated? Q 8. Were outcomes assessed in a standard, valid and reliable way for cases and controls? Q 9. Was the exposure period of interest long enough to be meaningful? Q 10. Was appropriate statistical analysis used?
JBI critical appraisal checklist for Cross-sectional studies:
Q1. Were the criteria for inclusion in the sample clearly defined? Q2. Were the study subjects and the setting described in detail? Q3. Was the exposure measured in a valid and reliable way? Q4. Were objective, standard criteria used for measurement of the condition? Q5. Were confounding factors identified? Q6. Were strategies to deal with confounding factors stated? Q7. Were the outcomes measured in a valid and reliable way? Q8. Was appropriate statistical analysis used?

dous effort to shed insight into taxonomic profiling, the core community members colonizing the urinary tract have not been understood. Nowadays, a growing body of literature has revealed that benign urological disorders are associated with compositionally different microbial ecosystems in the urinary tract(5). Bladder cancer (BC) is the fourth most frequent cancer in males and relatively common in females. Each year, roughly half a million new cases are diagnosed with BC, and more than 200000 individuals die globally(6). Substantial geographical variations have been demonstrated in the incidence of BC, with overall higher rates in the developed countries. The likelihood of getting BC increases with age, genetic susceptibility, tobacco smoking, chronic inflammatory conditions, and radiation exposure(7,8). Of interest, the characteristics of urinary tract microbial communities might be affected by the risk factors mentioned above. Therefore, these findings have led researchers to hypothesize that there might be a missing link between the development of BC and changes in the urinary microbiota.

Review question/objective

The quantitative objective was to identify the recent data on the role of urinary microbiota in BC.

MATERIALS AND METHODS

The proposed systematic review was conducted in accordance with critical review according to the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) statement, and the Joanna Briggs Institute methodology for systematic reviews.

Inclusion criteria

Types of participants

The quantitative component of this review was considered as patients with BC.

Types of intervention(s)

The quantitative component of the review was entailed studies that evaluated the urinary microbiota in BC patients.

Types of Comparison

This review's quantitative component was considered the urinary microbiota in patients without BC.

Types of outcomes

This review was considered studies that included the microbiota diversity as well as phylum and genus in patients with BC versus non-cancerous controls.

Types of studies

The quantitative component of the review was observational studies, including cohort, case-control, and cross-sectional studies.

Exclusion criteria

We excluded reviews, study protocols, letters to editors, case reports, editorials, dataset studies, brief correspondence, preprints, and articles that were not published in either English or Persian. We also conducted a search through the references of all the included papers

Table 2. The included studies' characteristics.

Author	BC	Control	Diversity		Phyla		Genera	
Hai Bi	29	26	Alpha-diversity	↑BC group (*P*<0.05).	*Firmicutes*, *Actinobacteria*, *Proteobacteria* and *Bacteroidetes*) in 94.4% cases		*Streptococcus*, *Bifidobacterium*, *Lactobacillus*, *Veillonella* and *Actinomyces*	
					BC	*Tenericutes* and *Proteobacteria* ↑ (P<0.05)	BC	*Actinomyces* *Actinomyces europaeus*
							Control	Others
Bassel Mansour	10	-	*N/M*		Urine	*Firmicutes* with abundance of 33%, followed by *Proteobacteria* (29%), *Actinobacteria* (23%), *Cyanobacteria* (7%) and *Bacteroidetes* (4%).	Urine	*Lactobacillus*, *Corynebacterium*, *Streptococcus* and *Staphylococcus*
					Tissue	*Firmicutes* (34%), *Actinobacteria* (23%), *Proteobacteria* (22%), *Bacteroidetes* (15%) and *Cyanobacteria* (8%).	Tissue	*Bacteroides*, *Akkermansia*, *Klebsiella* and *Clostridium* sensu stricto.
Suchitra K. Hourigan	22	-	Alpha-diversity	No significant difference between collection methods or sex.	Voided sample	*Firmicutes*↑	Cystoscopy sample	*Stenotrophomonas* ↑
			Beta-diversity	No significant difference between collection methods in all samples Significant difference in male when split the sex in collection method	Males	*Proteobateria*↑	Males	*Tepidimonas*↑
Juan Chipollini	27 (15 MIBC and 12 NMIBC)	10	Alpha-diversity	Significantly higher species evenness when compared to invasive (P = 0.031) and superficial tumors (P = 0.002). Significant differences in alpha diversity were observed by measurement of diversity evenness and richness within samples.	BC	*Bacteroides* and *Faecalbacterium*	*N/M*	
			Beta-diversity	No pairwise cohorts showed significant differential clustering.	Control	*Bacteroides*, *Lachnoclostridium*, *Burkholderiaceae*		
Jiarong Zeng	62 males (51 NMIBC and 11 MIBC)	19	Alpha-diversity	Bacterial richness indices (Observed Species index, Chao1 index, Ace index; all *P* < 0.01) increased in cancer group when compared with non-neoplastic group, while there were no differences in Shannon and Simpson index between two groups. Higher alpha diversity was observed in RE group when compared with NR group. Patients with higher alpha diversity tend to have a higher chance of recurrence compared with the men who had a lower diversity index.	- *Firmicutes* (RE: 28.3%, NR: 37.7%, p = 0.59) - *Proteobacteria* (RE: 10.2%, NR: 25.8%, p = 0.02) - *Actinobacteria* (RE: 6.7%, NR: 6.7%, p = 0.95) - *Bacteroidetes* (RE: 5.8%, NR: 6.4%, p = 0.89)		- *Staphylococcus* (RE: 11.9%, NR: 5.2%, p = 0.76) - *Streptococcus* (RE: 8.7%, NR: 10.3%, p = 0.94) - *Prevotella* (RE: 5%, NR: 4.1%, p = 0.95) - *Corynebacterium*_1 (RE: 4.5%, NR: 1.6%, p = 0.8) - *Lactobacillus* (RE: 0.04%, NR: 5.4%%, p = 0.06)	
Ahmed A.Hussein	43 (29 NMIBC and 14 MIBC)	10	Alpha-diversity	No significant difference between BC, controls, or NMIBC, and MIBC or between BCG responders vs. non-responders	NMIBC	*Proteobacteria*	NMIBC	*Cupriavidus* (P < 0.0007
			Beta-diversity	↑ in BC: *Actinomyces*, *Achromobacter*, *Brevibacterium*, and *Brucella* No significant difference between NMIBC, and MIBC or between BCG responders vs. non-responders	MIBC	*Firmicutes* *Proteobacteria*	MIBC	*Haemophilus* (P < 0.0001) and *Veillonella* (P < 0.0001)
					BCG responders	*Firmicutes* and *Proteobacteria*	BCG responders	- *Serratia* (P < 0.0001) - *Brochothrix* (P = 0.008) - *Negativicoccus* (P=0.006) - *Escherichia-Shigella* (P = 0.004) - *Pseudomonas* (P = 0.004)

Filippo Pederzoli	49	59	Alpha-diversity	No significant difference between BC patients and controls	*N/M*			*N/M*		
			Beta-diversity	No significant difference between BC patients and controls except between female cohorts in urine sample only						
Viljemka Bucevic Popovic	12 males	11	Alpha-diversity	No significant difference between BC patients and controls	*Firmicutes*, *Actinobacteria*, *Bacteroidetes* and *Proteobacteria*			*Streptococcus*, *Prevotella*, *Peptoniphilus*, *Campylobacter*, *Veillonella*, *Anaerococcus*, *Finegoldia*, and genus *1–68*, belonging to the *Tissierellaceae* family.		
								BC	Eight OTUs were enriched in urine of bladder cancer patients including the *Fusobacterium*, *Actinobaculum*, *Facklamia* and *Campylobacter* genera	
								Control	Five OTUs were enriched in healthy samples from the genera *Veillonella*, *Streptococcus*, *Corynebacterium*	
Peng Wu	26 NMIBC 5 MIBC	18	Alpha-diversity	No significant association with tumor grade	BC	*Proteobacteria* (39.7%) *Firmicutes* (32.8%) *Actinobacteria* (7.0%) *Bacteroidetes* (3.9%)		BC	↑*Acinetobacter*, *Anaerococcus*, *Rubrobacter*, *Sphingobacterium*, *Atopostipes*, *Geobacillus* (P<0.05)	
					Control	*Proteobacteria* (49.0%) *Firmicutes* (28.1%) *Actinobacteria* (6.2%) *Bacteroidetes* (9.4%)		Control	↑*Serratia*, *Proteus*, *Roseomonas*, *Ruminiclostridium-6*, and *Eubacterium–xylanophilum* (P<0.05)	
Bianca Oresta	51 males	10	Alpha-diversity	↑ in BC No difference with collection method	*Firmicutes*, *Actinobacteria*, *Bacteroidetes* and *Proteobacteria* were found dominating in both groups.			BC	↑ *Veillonella* (p=0.04) and *Corynebacterium* (p=0.03) ↓*Ruminococcus* (p=0.03) and of an unclassified genus of *Enterobacteriaceae* (p=0.025)	
									↑*Veillonella* in pTa/T1 HG, CIS and T2 tumors compared to controls and pTa LG tumors ↑*Corynebacterium* and *Staphylococcus* in HG NMIBC and pTa LG tumors ↓*Bifidobacterium* (p=0.02) characterized T2	
			Beta-diversity	No difference between BC and control groups or with collection method	Midstream	↑*Firmicutes* (p <0.0001) and *Fusobacteria* (p <0.0001) ↓*Actinobacteria* (p=0.009) and *Firmicutes* (p=0.015)		Midstream	↑*Streptococcus* (p <0.0001), *Enterococcus* (p <0.0001), *Corynebacterium* (p=0.038) and *Fusobacterium* (p<0.0001) ↓*Ruminococcaceae* (unknown genus) (p <0.0001)	
					Catheter	*BC: ↑Corynebacteriaceae* Control: ↑*Enterobacteriaceae*		Catheter	*N/M*	
Bassel Mansour	55	12 (Prostatic Hyperplasia) 34 (Healthy volunteers)	Alpha-diversity	The tumor-specific microbiome was still significantly lower than that of non-tumor samples (p < 0.001).	Median abundance of bacteria belonging to the Cyanobacteria phylum was significantly higher in the BC group (p = 0.011).			Although Oxyphotobacteria (from the Cyanobacteria phylum) did not belong to the predominant bacterial genus in the microbiome, there was significant difference in their abundance between the BC and PH groups (2.11% vs. 0.07%; p = 0.024).		
			Beta-diversity	Showed significant difference between the two cohorts (p = 0.001).	BC	*Firmicutes (46%)* *Proteobacteria (23%)* *Actinobacteria (13%)* *Bacteroidetes (11%)*	p > 0.05 p = 0.006 p < 0.001 p < 0.001	BC	*Staphylococcus (7.89%)* *Corynebacterium (3.83%)* *Faecalibacterium (1.92%)* *Bacteroides (3.22%)*	p < 0.001 p = 0.001 p < 0.001 p < 0.001
					PH	*Firmicutes (45%)* *Proteobacteria (16%)* *Actinobacteria (4%)* *Bacteroidetes (30%)*		PH	*Staphylococcus (0.59%)* *Corynebacterium (0.63%)* *Faecalibacterium (7.79%)* *Bacteroides (21.54%)*	
Guoqin Mai	24 (18 males and 6 females)	-	*N/M*		*Proteobacteria*, *Firmicutes*, *Actinobacteria*, *Tenericutes*, and *Bacteroidetes*			*Enterobacteriaceae-g-* , *Streptococcus*, *Lactobacillus*, *Ureaplasma*, *Corynebacterium*, *Stenotrophomonas*, *Enterococcus*, and *Staphylococcus*		
Yifeng Qiu	40 males	-	Alpha-diversity	↑RE than NR. - Shannon Index: p=0.0007 - Simpson Index: p=0.0004 - Observed Species Index: p=0.046 - Ace Index: p=0.108 - Chao1 Index: p=0.192	RE (n=12)	*Firmicutes* *Proteobacteria* *Actinobacteria* *Bacteroidetes*		RE	*Staphylococcus* *Streptococcus* *Escherichia_Shigella* *Prevotella* *Acinetobacter* *Lactobacillus* - Compared to the NR group, patients in the RE group had increased abundance of *Pseudomonas* at the genus level (FDR adjusted p-value= 0.0334)	
			Beta-diversity	PCoA scatter plots showed two	NR (n=28)			NR	*Streptococcus* *Escherichia_Shigella*	

				obviously distinct clusters between the RE and NR groups. - Weighted Unifrac: p=0.02 - Unweighted Unifrac distance: p=0.001 - Bray–Curtis distance: p=0.055				*Lactobacillus* *Prevotella* *Staphylococcus* *Acinetobacter*
Wenchao Ma	15 males	11 males	Alpha-diversity	Species richness: P = 0.24 Simpson index: P = 0.069	There are only nine different phyla between the healthy people and the bladder cancer patients. *Dependentiae* *Zixibacteria* *Latescibacteria* *Halanaerobiaeota* *Cloacimonetes* *Entotheonellaeota* *Rokubacteria* *Gemmatimonadetes* *Nitrospirae*		There are only 10 different genera between the healthy people and the bladder cancer patients. *Sphingomonas* *Clostridium_sensu_stricto_13* *Flavisolibacter* *Fonticella* *Geobacter* *Sporacetigenium* *Marmoricola* *Ellin6067* *Bryobacter* *Paenisporosarcina*	
			Beta-diversity	Adonis test, Bray-Curtis: P < 0.03				
Fei Liu	22 tissues (17 MIBC, 5 NMIBC)	12 tissues	Alpha-diversity	Shannon: p=0.0417, demonstrating a significantly lower diversity in cancerous tissues compared to noncancerous tissues.	All	*Proteobacteria (54.1%)* *Firmicutes (23.7%)* *Bacteroidetes (13.4%)* *Actinobacteria (4.4%)*	All	*Cupriavidus (16.9%)* *unclassified Brucellaceae (6.0%)* *Ralstonia (5.5%)* *Lactobacillus (5.3%)*
			Beta-diversity	Weighted UniFrac (ADONIS P<0.001) between cancerous and noncancerous tissues.	BC	↑ *Proteobacteria spp* and *Actinobacteria spp*	BC	↑ *Cupriavidus spp, unclassified Brucellaceae, Acinetobacter, Escherichia-Shigella, Sphingomonas, Pelomonas, Ralstonia, Anoxybacillus,* and *Geobacillus*
					Control	↑ *Firmicutes* and *Bacteroidetes*	Control	↑ *Lactobacillus, Prevotella_9,* and *Ruminococcaceae*
Chuan Qin	32 6 MIBC, 26 NMIBC (21 males and 11females)	15 (10 males and 5 females)	*N/M*		*N/M*		BC (GI microbiota)	*Streptococcus* *Escherichia* *Bacteroides* *Akkermansia* *Ruminococcus* *Parabacteroides* *Butyricicoccus* *Fusobacterium* *Veillonella* *Phascolarctobacterium* *Paraprevotella* *Collinsella* *Lachnoclostridium* *Bilophila*
							Control (GI microbiota)	*Bifidobacterium* *Lactobacillus* *Prevotella* *Roseburia* *Megamonas* *Faecalibacterium* *Clostridium* *Oscillibacter* *Romboutsia* *Eubacterium* *Subdoligranulum* *Fusicatenibacter* *Ruminococcus* *Coprococcus* *Megasphaera* *Sutterella* *Blautia* *Anaerostipes* *Dialister* *Gemmiger* *Dorea*
Mónica Parra-Grande	32 (27 males and 5 females)	-	Alpha-diversity	Indices were higher in the non-tumor mucosa than in the tumor mucosa, with statistical significance detected at the phylum and genus level.	Matched tumor tissue (n=26)	*Firmicutes= 40.16%* *Bacteroidetes= 29.15%* *Proteobacteria= 22.96%* *Actinobacteria= 6.18%*	Matched tumor tissue (n=26)	*Bacteroides=16.66%* *Escherichia-Shigella=6.63%* *Staphylococcus=6.06%* *Enterococcus=5.18%* *Alistipes=4.45%* *Phascolarctobacterium=4.42%* *Prevotella=4.28%* *Parabacteroides=4.13%* *Propionibacterium=2.91%*

			Beta-diversity	At the phylum and genus level, no differences were observed between the two groups	Non-tumor tissue (n=26)	*Firmicutes= 39.56%* *Bacteroidetes= 28.17%* *Proteobacteria= 21.35%* *Actinobacteria= 9.05%*	Non-tumor tissue (n=26)	*Bacteroides=16.27%* *Escherichia-Shigella=6.28%* *Phascolarctobacterium=5.19%* *Propionibacterium=4.49%* *Staphylococcus=4.42%* *Prevotella=4.32%* *Parabacteroides=3.81%* *Alistipes=3.25%* *Enterococcus=1.59%*
Jian-Xuan Sun	22	-	Alpha-diversity	The NMIBC group was significantly superior to the MIBC group. - Chao1, Shannon, and Simpson: $P<0.05$	MIBC (n=15)	*Proteobacteria=68.45%* *Firmicutes=14.76%* *Actinobacteriota=12.98%* *Firmicutes_A=2.4%*	MIBC (n=15)	*Ralstonia=56.29%* *Cutibacterium=9.82%* *Enterococcus=6.91%* *Sphingomonas=5.77%* *Metamycoplasma=4.60%*
			Beta-diversity	Bray-Curtis: $P<0.001$ Binary Jaccard: $P<0.001$ Euclidean: $P<0.01$	NMIBC (n=7)	*Proteobacteria=39.09%* *Firmicutes=19.17%* *Actinobacteriota=14.92%* *Firmicutes_A=13.13%* *Bacteroidota=11.55%*	NMIBC (n=7)	*Ralstonia=22.16%* *Cutibacterium=6.60%* *Bacteroides=5.51%* *Staphylococcus=5.27%* *Acinetobacter=5.07%*
Yuhang Zhang	51 NMIBC	47	Alpha-diversity	Chao1 index ($p<0.001$) and rank abundance curves showed that α diversity of the urinary bacterial community of NMIBC group was lower than those of the healthy control groups.	NMIBC	*Eubacterium sp. CAG:581* *Bacteroides sp. 4_3_47FAA* *Flavobacteriales*	*N/M*	
					Control	*N/M*		
			Beta-diversity	Bray-Curtis: $p=0.025$ Binary Jaccard: $p=0.003$				
Muhammed Selcuk Ozer	12	-	*N/M*		First voided urine	*Firmicutes=32.54%* *Proteobacteria=32.44%* *Bacteroidetes=13.88%* *Actinobacteria=11.28%* *Tenericutes=4.8%*	First voided urine	*Escherichia-Shigella:8.54%* *Streptococcus: 4.44%* *Ureaplasma:4.06%* *Corynebacterium:2.96%* *Stenotrophomonas:2.66%* *Lactobacillus:2.65%* *Staphylococcus:1.42%*
					Midstream urine	*Firmicutes=33.79%* *Proteobacteria=31.14%* *Bacteroidetes=16.59%* *Actinobacteria=11.87%* *Tenericutes=4.56%*	Midstream urine	*Escherichia-Shigella:6.90%* *Streptococcus:3.21%* *Ureaplasma:3.98%* *Corynebacterium:2.88%* *Stenotrophomonas:3.04%* *Lactobacillus:3.18%* *Staphylococcus:1.13%*
					Statistical differences were not significant.		Statistical differences were not significant.	
Jan Hrbáček	34	29 (upper urinary tract stone disease, n = 21; benign prostate hyperplasia, n = 8)	Alpha-diversity	BC patients had lower UM richness and diversity than controls. - Number of observed OTUs: $p=0.015$ - Shannon: $p=0.049$ - ACE: $p=0.978$ - Chao1: $p=0.499$ - Simpson: $p=0.110$	*Proteobacteria (46.7 ± 31.7%), Firmicutes (20.5 ± 21.7%), Bacteroidetes (12.8 ± 15.0%)* and *Actinobacteria (10.9 ± 12.4%)*		BC	*Pseudomonas: 11.6±18.4%* *Campylobacter: 7.2±13.2%* *Ralstonia: 7.2±16.4%* *Veillonella: 5.1±20.4%* *Staphylococcus: 3.4±6.7%* *Ezakiella: 3.3±8.1%* *Streptococcus: 3.0±9.0%* *Porphyromonas: 2.3±4.1%* *Anaerococcus: 2.1±6.4%* *Atopobium: 1.7±9.5%*
					BC	*Proteobacteria* *Firmicutes ↑* *Bacteroidetes ↑* *Actinobacteria ↑*		
			Beta-diversity	PERMANOVA performed on the data set showed that the observed differences in these OTUs were statistically significant: $R = 0.029$, $P = 0.039$	Control	*Proteobacteria ↑* *Firmicutes* *Bacteroidetes* *Actinobacteria*	Control	*Pseudomonas: 12.8±19.1%* *Staphylococcus: 5.6±11.8%* *Ralstonia: 4.8±9.4%* *Cutibacterium: 3.5±4.2%* *Veillonella: 3.3±11.5%* *Moraxella: 2.3±2.7%* *Porphyromonas: 2.3±5.6%* *Campylobacter: 2.2±6.4%* *Streptococcus: 2.0±4.6%* *Corynebacterium: 1.6±1.5%*
Filippo Pederzoli	42	-	Alpha and beta diversity metrics did not differ between responders and nonresponders.		GI Microbiota		GI Microbiota	
					Responders (n=23)	*Proteobacteria*	Responders	*Sutterella*
					Non-responders (n=19)	*NM*	Non-responders	*Ruminococcus bromii spp.*
Carmela Nardelli	48 (TURBT)	43	Alpha-diversity	Chao1 did not show significant difference.	*N/A*		First-Morning Voiding	
							TURBT	*Actinobaculum: 4.68%* *Lactobacillus: 3.92%* *Mobiluncus: 1.19%* *Peptoniphilus: 6.38%* *Porphyromonas: 3.69%* *Propionimicrobium: 3.39%* *Streptococcus: 2.32%*
			Beta-diversity	PERMANOVA $p=0.001$			Control	*Actinobaculum: 1.72%* *Lactobacillus: 10.29%* *Mobiluncus: 0.50%* *Peptoniphilus: 2.59%* *Porphyromonas: 0.37%* *Propionimicrobium: 0.92%* *Streptococcus: 6.17%*

<table>
<tr><td rowspan="5">Yuchao Liu</td><td rowspan="5">32</td><td rowspan="5">5</td><td rowspan="4">Alpha-diversity</td><td rowspan="4">There was no statistically significant difference in ACE, Chao1, Shannon, observed species or Simpson index among groups.</td><td colspan="3">Midstream Urine</td><td colspan="4">Midstream Urine</td></tr>
<tr><td rowspan="3">BC</td><td>Studer (n=13)</td><td><i>Proteobacteria: 35.9%</i>
<i>Firmicutes: 30.5%</i>
<i>Bacteroidota: 26.4%</i></td><td rowspan="3">BC</td><td>Studer (n=13)</td><td colspan="2"><i>Enterococcus: 13.1%</i>
<i>Escherichia-Shigella: 18.4%</i>
<i>Barnesiella: 14.4%</i></td></tr>
<tr><td>Bricker (n=10)</td><td><i>Proteobacteria: 50.9%</i>
<i>Firmicutes: 31.0%</i>
<i>Bacteroidota: 11.9%</i></td><td>Bricker (n=10)</td><td colspan="2"><i>Enterococcus: 14.4%</i>
<i>Escherichia-Shigella: 12.7%</i>
<i>Barnesiella: 5.3%</i></td></tr>
<tr><td>Cutaneous Ureterostomy (n=9)</td><td><i>Proteobacteria: 67.9%</i>
<i>Firmicutes: 23.2%</i>
<i>Bacteroidota: 4.8%</i></td><td>Cutaneous Ureterostomy (n=9)</td><td colspan="2"><i>Enterococcus: 24.0%</i>
<i>Escherichia-Shigella: 14.4%</i>
<i>Barnesiella: 1.3%</i></td></tr>
<tr><td>Beta-diversity</td><td>Overall, the PERMANOVA test showed that the observed difference was statistically significant (Adonis test, Binary-Jaccard, F=1.4795, P<0.001).</td><td colspan="2">Control</td><td><i>Proteobacteria: 15.5%</i>
<i>Firmicutes: 41.8%</i>
<i>Bacteroidota: 31.2%</i></td><td colspan="2">Control</td><td colspan="2"><i>Enterococcus: 3.7%</i>
<i>Escherichia-Shigella: 0.1%</i>
<i>Barnesiella: 10.2%</i></td></tr>
<tr><td rowspan="3">Christopher James</td><td rowspan="3">29 High-grade NMIBC (22 males, 7 females)</td><td rowspan="3">-</td><td rowspan="3">Alpha-diversity</td><td rowspan="3">Alpha (within sample) diversity of the entire cohort, the bacterial richness (the estimated number of taxa) decreased significantly over time (P=0.01). The Shannon and Simpson indices differed significantly (P=0.02 and 0.01 respectively) with patients who recurred having more diverse bladder microbiomes than those who did not recur.</td><td colspan="3" rowspan="3"><i>NM</i></td><td colspan="4"><i>Relative Abundance (mean)</i></td></tr>
<tr><td colspan="2">RE (n=8)</td><td><i>Aerococcus: 0.65</i>
<i>Escherichia/Shigella: 0.51</i>
<i>Ureaplasma: 0.10</i></td><td rowspan="2"><i>p <0.01</i>
<i>p=0.05</i>
<i>p=0.01</i></td></tr>
<tr><td colspan="2">NR (n=21)</td><td><i>Aerococcus: 0.36</i>
<i>Escherichia/Shigella: 0.68</i>
<i>Ureaplasma: 0.29</i></td></tr>
<tr><td rowspan="2">Ahmed A. Hussein</td><td rowspan="2">68 (58 males and 10 females)</td><td rowspan="2">-</td><td>Alpha-diversity</td><td>Not significantly different.
- Observed Index: p=0.52
- Chao1 Index: p=0.77
- Shannon Index: p=0.12
- Simpson Index: p=0.17</td><td colspan="3" rowspan="2"><i>NM</i></td><td colspan="2">RE (n=42)</td><td colspan="2"><i>Veillonella, and Bifidobacterium ↑ (> 2FC, p = 0.0002)</i></td></tr>
<tr><td>Beta-diversity</td><td>Significantly different.
- Bray Curtis Index: p=0.037</td><td colspan="2">NR (n=26)</td><td colspan="2"><i>Escherichia-Shigella (> 2FC, p = 0.0002) and Halococcus (> 2FC, p = 0.0008) ↑</i></td></tr>
<tr><td rowspan="2">Vitor Heidrich</td><td rowspan="2">32 males (NMIBC)</td><td rowspan="2">41 (BPH)</td><td>Alpha-diversity</td><td>Not significantly different.
- ASV richness: p=0.87
- Faith's PD: p=0.77
- Gini-Simpson: p=0.63
- Shannon: p=0.81</td><td colspan="3" rowspan="2"><i>NM</i></td><td colspan="4" rowspan="2">They were unable to identify differentially abundant genera between groups.</td></tr>
<tr><td>Beta-diversity</td><td>Not significantly different.
- Weighted UniFrac: p=0.66</td></tr>
</table>

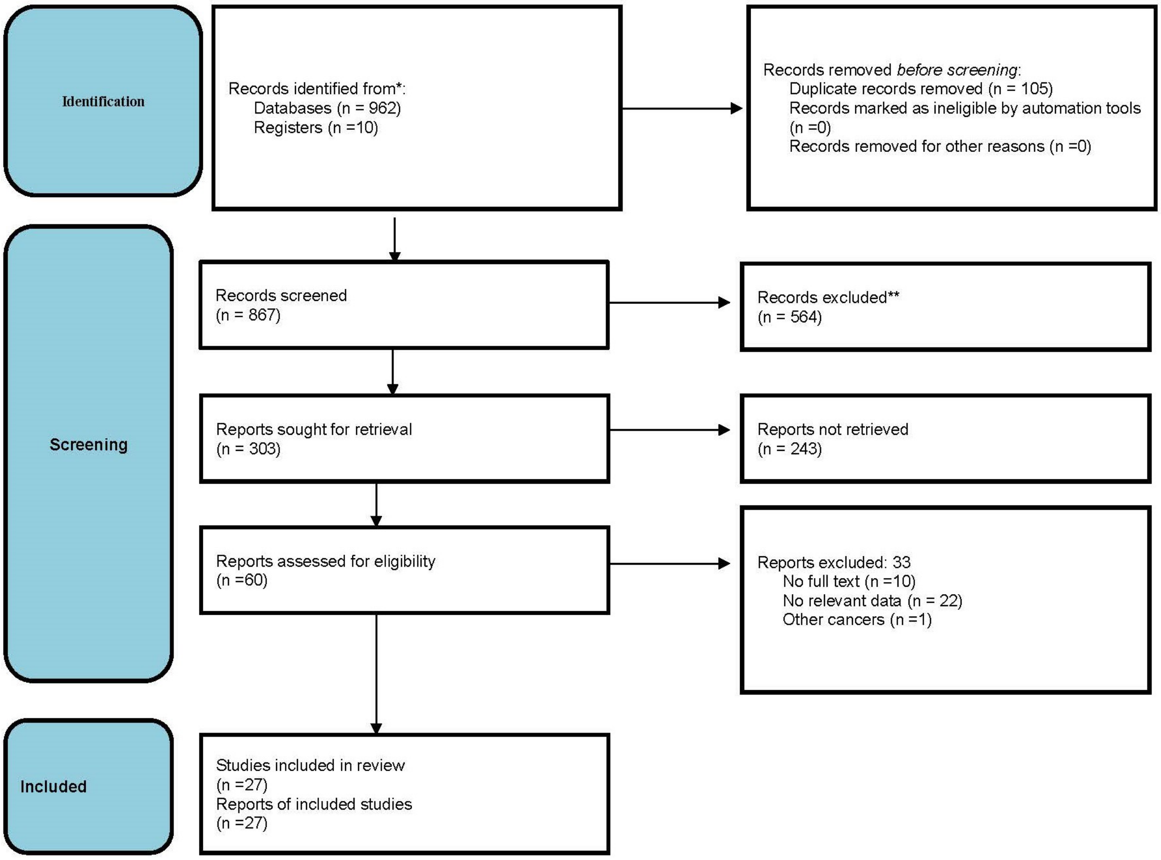

Figure 1. Study flow diagram

to identify any further studies of relevance.

Search strategy

The search strategy aims to find both published and unpublished studies. A three-step search strategy was utilized in this review. An initial limited search of Pubmed was undertaken, followed by analyzing the textwords, including the title and abstract, and the index terms. Afterward, the second search using all identified keywords and index terms was undertaken across all included databases until January 2024. MEDLINE (via OVID), PubMed, Embase (www.embase.com), Cochrane Library, Cinahl (via Ebsco), Web of Science, and Scopus were searched. Google Scholar were searched for any additional related articles. The main search terms include but are not limited to ‘Microbiota’, ‘Microbiome’, ‘Microflora’, ‘Urinary Bladder Neoplasm’, ‘Bladder cancer’. The search strategy includes both free text-words and controlled vocabularies related to selected keywords. The search strategy of Medline (via OVID) is available in Appendix I. Thirdly, the reference lists of all identified reviews and articles were searched for additional studies. Studies published in any language and any date were considered for inclusion in this review.

Study selection

Following the search, all identified citations were collated and uploaded into EndNote, and duplicate studies were removed. Two independent reviewers screened titles and abstracts for assessment against the inclusion criteria for the review. Potentially relevant studies were retrieved in full, and their citation details were imported into the Joanna Briggs Institute System for the Unified Management, Assessment and Review of Information (JBI SUMARI) (Joanna Briggs Institute, Adelaide, Australia). The full texts of selected citations were assessed in detail against the inclusion criteria by two independent reviewers. Reasons for excluding full-text studies that do not meet the eligibility criteria were recorded and reported in the systematic review. Any disagreements between the reviewers were resolved through discussion or consulting with a third reviewer. The search results were reported in full in the final systematic review and presented in a Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) flow diagram.

Assessment of methodological quality

Two reviewers conducted independent assessments of publications with quantitative data to assess their methodological validity using the Joanna Briggs Institute critical appraisal tool for systematic review and meta-analysis. Any discrepancies between the reviewers were resolved through discussion or by consulting a third reviewer. The studies selected were categorized into three quality levels based on their scores: those with a total score above 80% were considered high quality, scores ranging from 60% to 80% were classified as medium quality, and scores below 60% were designated as low quality.

Data collection

Data extraction from included studies was performed by two independent reviewers using the modified standardized JBI data extraction tool. Specific details were extracted from included studies include first author, publication year, study design, country, type of implant, age, outcome, and sample size. Any disagreement among all reviewers was resolved by discussion.

Study inclusion

During the electronic search, hand search, and reference check, we identified 972 citations. After omitting duplicated citations, 867 studies remained for the screening process. Sixty studies were selected based on titles and abstracts. In the full-text selection step, 33 studies were excluded. Finally, 27 studies were included for the critical appraisal process and included in this study. Additional information on the selection process and the reason for excluding studies are presented in the PRISMA flow chart (**Figure 1**).

Methodological quality

JBI appraisal checklist was used to assess possible biases. The results of eligible studies' evaluation are presented in **Table 1**. According to the results, all of the studies achieved more than 60% of score, and represent that they had good quality.

RESULTS

Characteristics of included studies and findings

A total of 27 studies comprising 1338 individuals were included for the systematic review. Among the included studies, 926 were BC patients and 412 were controls. Fifteen studies compared the microbiota of BC patients with that of control subjects, while other 12 studies included only BC patients with MIBC or NMIBC. The oldest studies were published in 2018 (n = 2), three studies in 2019, five studies in 2020, four ones in 2021, four articles in 2022, five in 2023, and the latest were published in 2024 (n = 4).

The most common sample collection method was midstream urine (n = 9 studies). In seven studies, the sample collection method was TURB or surgery; in four studies, it was collected via catheterization of the urinary tract. In one study, samples were collected through both voided urine and cystoscopy. Two studies utilized fecal samples, one compared first-voided and midstream urine in each individual, one study took both urine and TURBT samples, and another collected urine samples either during cystoscopy/TURBT or through midstream urine specimens. Nineteen articles used 16S rDNA sequencing, while the remaining articles utilized the 16S rRNA molecule.

In the majority of studies, there were reported significant differences in alpha diversity in patients with BC compared to the healthy controls or between NMIBC and MIBC, or across tumor grades. The characteristics of phyla genera with splitting in BC including NMIBC and MIBC, as well as healthy controls and responders versus non-responders to BCG therapy are summarized in **Table 2**.

Review findings

In a study by Bi et al.(9), the clean urine samples of 29 BC patients (median and range of 67 (48–88) years) and 26 control subjects (median and range of 56.5 (28–82) years) were gathered and analyzed by amplicon-based NGS approach. These data indicated that 94.4% of microbiome phyla in all cases consisted of Firmicutes, Actinobacteria, Proteobacteria, and Bacteroidetes, respectively. Corynebacterium, Staphylococcus, Lactobacillus, Streptococcus, and Prevotella were the most frequent genera among them.

Intergroup comparison revealed that BC patients had different microbiome communities. Alpha diversity was, however, significantly higher in BC patients compared to healthy controls($P < 0.05$). In terms of phyla, Proteobacteria and Tenericutes had statistically significantly higher abundance in BC patients and 26 genera had significant intergroup abundance differences. Overall, Streptococcus, Actinomyces, Lactobacillus, Bifidobacterium, and Veillonella were widely present in both groups. While the last three probiotics were significantly abundant in the control group, Actinomyces were more frequent in the BC group, suggesting a potential role for A. europaeus in BC development.

Hourigan et al.(10), in their studies on 22 patients with non-muscle invasive BC (NMIBC), compared microbiome diversity resulting from 16 SrRNA gene sequencing between different sample gathering methods, including cystoscopy-mediated, midstream, and voided urine samples. There was no significant difference in beta diversity concerning sampling methods except for voided and cystoscopy samples in males ($p = 0.006$). Regarding alpha diversity, no differences were seen through the methods mentioned above.

Mansour et al.(11) aimed to compare microbiota composition between BC tissues removed by transurethral resection and urine samples of the same patients. Five males and five females with a mean age of 63.9 years and BC (MIBC/NMIBC = 4/6) were enrolled. Firmicutes (34% in tissue, 33% in urine) was the most common phyla in both sampling techniques. Proteobacteria, Actinobacteria, Cyanobacteria, and Bacteroidetes were also among the most abundant phyla in either group. When it came to genera levels, Akkermansia, Bacteroides, Clostridium sensustricto, Enterobacter, and Klebsiella were considerably higher in tissue samples. The latter finding supports the fact that urine microbiota cannot fully represent cancerous tissue microbiota. Furthermore, there was no significant difference between the microbiota of patients with MIBC compared to those with NMIBC.

Another study comprising 37 subjects, including 10 healthy individuals, 15 MIBC patients, and 12 NMIBC patients evaluated and characterized the urinary microbiome of patients with BC. The results illustrated that the species evenness and richness of the species were higher in the control group compared to those in the BC group ($P < 0.05$). Interestingly, Bacteroides, Lachnoclostridium, and Burkholderiaceae were significantly enriched in the control samples. In invasive cancer samples, Bacteroides and Faecalbacterium showed significant enrichment(12).

Zeng et al.(13) examined the alterations in the urinary microbiome and explored their contribution to clinical outcomes in 62 male patients with BC versus 19 controls. The study comprised 40 patients diagnosed with NMIBC who underwent transurethral resection of bladder tumor and were on follow up. Prior to cystoscopy, midstream urine samples were gathered. The median follow-up time was 12 (5.25–25) months with 12.5% of patients experiencing recurrence but no progression to MIBC. The abundance of nine genera, including Brachybacterium and Micrococcus were higher in those who developed tumor recurrence. Alpha diversity was significantly higher in the patients experiencing disease recurrence group compared to that in those not experiencing disease recurrence. Moreover, they found that the cancer group had remarkably higher species richness indices (Observed Species, Chao1, and Ace) compared to healthy controls ($P < 0.01$). The authors suggested that these indices could serve as diagnostic markers for BC based on the results of their ROC curve (overall 80% area under the curve).

Hussein et al.(14) compared the urinary microbiome composition in terms of alpha and beta diversities in 43 patients with BC (transurethrally obtained sample) and 10 healthy controls (midstream catch). BC patients had an average age of 70 years, seven were female, and 29 suffered NMIBC. Out of 29 patients with NMIBC, 11 were treated with Bacillus Calmette Guerin (BCG). After a median 13 months of follow-up, six patients experienced recurrence. Although the beta diversity was remarkably different between patients with cancer and control subjects, there was no difference between MIBC and NMIBC patients, and there was no association with response to BCG. Considering genera, Actinomyces, Brucella, Brevibacterium, Achromobacter were significantly more common in the BC patients compared to controls. In NMIBC, Cupriavidus was significantly more abundant, while in MIBC patients, Hemophilus and Veillonella were significantly more common in the urine. In responders to BCG, Serratia, Pseudomonas, Escherichia-Shigella, Negativicoccus, and Brochothrix were significantly more abundant when compared to non-responders. Despite statistically significant differences in the abundance of some phyla and genera between males and females with BC, there was no difference in diversity metrics (alpha and beta).

Pederzoli et al.(15) performed a study that had the aim to assess sex-based differences in urinary and tissue-associated microbiome using 59 healthy individuals and 49 therapy-naïve BC patients. They found that Klebsiella was more abundant in the morning midstream voided urine of BC women compared to controls. In males however, there was no differently enriched genera. In terms of tissues samples, the genus Burkholderia was more abundant in the cancerous tissue of either sex, suggesting an association with BC development. Overall, diversity metrics (alpha and beta) were not significantly different between cancer and control groups, except the beta diversity of BC versus controls in females. Urine and tissue samples shared approximately 80% of bacterial communities.

Popović(16) compared the microbiome of 12 BC male patients with 11 healthy controls. Firmicutes, Actinobacteria, Bacteroidetes, and Proteobacteria were the most abundant phylum in either group. Although there was no difference in microbiome composition between the two groups, the genus Fusobacterium enrichment was remarkably higher in the cancer group.

Another study by Wu et al.(17) characterized the urinary microbial community in BC males (n = 31) and compared it with non-neoplastic controls (n = 18). The midstream urine was collected for DNA extraction followed by high throughput 16S rRNA sequencing. Bacterial richness indices, including Ace, Chao1, and Observed Species, were all remarkably higher in the cancer group. While Sphingobacterium, Acinetobacter, and Anaerococcus genera were enriched, Proteus, Roseomonas, and Serratia were decreased among BC patients. The beta diversity was significantly different between cancer and controls though the difference was not significant across tumor grades. Eventually, Bacteroides, Herbaspirillum, and Porphyrobacter were more abundant in those with an increased risk of disease recurrence.

Oresta et al.(18) determined the urinary microbial composition difference of 51 BC male patients and 10 healthy controls with different sample collection methods. In samples collected by a catheter from BC patients, a significant increment in Veillonella and Corynebacterium together with a decrement in Ruminococcus were observed in BC cases compared to controls. The abundance of Burkholderiaceae was significantly higher in bladder washout samples compared to catheter collected samples. Fusobacterium, Corynebacterium, Enterococcus, and Streptococcus were significantly more common in midstream urine samples. This study demonstrated that the sampling method influences the differential abundance of microbial communities.

Mansour et al.(19) evaluated human beta defensin (HBD) levels and bladder mucosal microbiota in 55 bladder cancer patients and 12 patients with prostate hyperplasia who underwent TUR, compared to 34 healthy individuals. They stated that HBD1 level was lower in cancer group, while HBD2,3 significantly were higher in this group. The most prevalent microbiota in tumor group were Staphylococcus, Corynebacterium, and Oxyphotobacteria, which were related to higher urinary HBD2,3 levels, whereas Faecalibacterium and Bacteroides were lower in tumor group. They found that microbiota and HBD levels should be considered in development, prognosis, treatment, and immunotherapy in bladder cancer, too.

Hrbacek et al.(20) evaluated urinary microbiome in 34 bladder cancer patients compared to 29 controls with benign urologic problems who underwent endoscopic evaluations. Richness and diversity of microbiome profile were lower in bladder cancer patients. Veillonella, Varibaculum, and Methylobacterium-Methylorubrum were the most prevalent taxa in bladder cancer group, whereas Pasteurella, Corynebacterium, and Acinetobacter were higher in controls.

Qin et al.(21) evaluated gut microbiota in 32 bladder cancer patients compared to 15 healthy individuals. They showed that Bifidobacterium, Lactobacillus, Streptococcus, Blautia, and Eubacterium were dysregulated in BC group. They revealed that gut dysbiosis was associated with metabolites which are potentially related to bladder cancer, such as increased cholesterol sulfate level and decreased 11-Z-eicosenoicacid, 3-methoxytyrosine, abrine, and etc. They showed that Clostridium-SP-CAG-590 was related to 11-Z-eicosenoic acid, arachidic acid, conjugated linoteic acid, elaidic acid, linoelaidic acid. These metabolites and Clostridium-SP-CAG-590 were decreased in bladder cancer patients. At genus level, Bifidobacterium, and Lactobacillus were prevalent in healthy individuals, while Streptococcus, and Escherichia were more abundant in bladder cancer group.

Parra Grande et al.(22) studied microbial profile in 32 patients with bladder cancer who underwent radical cystectomy. They compared tumoral versus non tumoral mucosa and found that alpha diversity was higher in non-tumoral mucosa, Actinobacteria were higher in non-tumoral samples, which emphasized that microbial profile can have a potential protective role against bladder cancer. They stated that microbial characterization differed depending on tumor grade. Enterococcus was enriched in low-grade tumors. Non tumoral samples contained higher microbial richness compared to bladder cancer samples. Bacteroides, Escherichia-Shigella, Staphylococcus, Enterococcus, Alistipes, and Parabacteroides genera were higher in bladder cancer samples. They could not find any association between the tissue microbial profile and clinical or pathological variables.

Liu et al. (23) assessed bladder tissue microbial diversity in bladder cancer patients. They evaluated 22 bladder cancer tissue samples. They stated that Cupriavidus spp., Brucellaceae, and Actinobacter had higher abundance in bladder cancer patients, while Lactobacillus, Prevotella-9, and Ruminococcaceae had lower abundances in these patients. The most abundant phyla were Proteobacteria, Firmicutes, and Bacteroidetes. Alpha diversity richness was significantly lower in cancerous samples, and significant differences were identified in beta diversity between cancerous and noncancerous tissues.

Ozer et al.(24) evaluated bacterial 16S rRNA in first voided versus mid-stream urine samples in 12 men with NMIBC (Ta low grade) to find the best non-invasive diagnostic method for microbiotal studies. They stated that they did not identify any significant differences between microbiota in mid-stream and first voided urine samples. Firmicutes were the most abundant phylum, and Gammaproteobacteria was the most prevalent class, and Corynebacteriaceae and Mycoplasmataceae were the most prevalent family.

Mai et al.(25) evaluated the most important microbiota in bladder cancer urine samples in 24 patients (18 males and 6 females). They noticed that Acinetobacter level was higher in bladder cancer patients compared to healthy individuals. Proteobacteria, Firmicutes, and Actinobacteria were the most abundant phyla in bladder cancer patients. Gammaproteobacteria, Bacilli, and Actinobacteria were the most abundant class in bladder cancer patients. They noticed that Acinetobacter might be considered as a potential urinary marker in bladder cancer patients.

Zhang et al.(26) introduced Eubacterium sp.CAG:581 as a potential marker for early diagnosis and prognosis of NMIBC. They noted that Eubacterium sp.CAG:581, Bacteroides sp.4-3-47FAA, and Flavobacteroides were more prevalent in NMIBC group. They stated that alpha diversity was lower in NMIBC patients, while beta diversity was differed in both groups. They determined the relationship between Eubacterium sp.CAG:581 and CM1/ERK1/ERK2 phosphorylation/MMP9 in promoting NMIBC growth.

Sun et al.(27) evaluated bladder tissue microbial characterization difference between MIBC and NMIBC patients using 2bRAD method. They demonstrated that microbial diversity was higher in NMIBC group. They found Ralstonia-sp 000620465 as the most prevalent microbiota in both groups. Acinetobacter, Anoxybacillus, Brevibacillus, and Staphylococcus were enriched in NMIBC, whereas Ralstonia_mannitolilytica, Ralstonia_pickettii, and Ralstonia_sp000620465 were more common in MIBC group.

Qiu et al.(28) investigated the 40 males (12 with recurrent NMIBC and 28 without recurrent NMIBC). They showed alpha diversity was higher in recurrent group, which was related to more elevated Ki-69 expression and shorter recurrence-free survival. Pseudomonas, Staphylococcus, Corynebacterium, and Acinetobacter were predominant in recurrent group due to Lefse analysis.

Abstract Reviews

Bukavina et al.(29) assessed gut microbiota in 29 bladder cancer patients underwent cystectomy, compared to control group. They revealed that Bacteroidales, Clostridiales, and Bulkholderiales were the most prevalent GI microbiota order in bladder cancer patients. There was no difference between in alpha diversity between bladder cancer and healthy individuals. They highlighted that overexpression of Campylobacter and Fusobacterium in bladder cancer patients which was not mentioned in previous articles.

Knorr et al.(30) (2022) investigated urinary microbiome in BCG responders and non-responders bladder cancer patients. They noticed that microbiota profile was different between 2 groups. Lactobacillus crispatus were abundant in BCG responders and Corynebacterium specious were higher in non-responders.

Houenstein et al.(31) investigated urinary microbiome profile in recurrent and non-recurrent bladder cancer patients after TURBT. They assessed 42 patients with recurrent bladder tumor and 26 non recurrent tumor. All patients in non recurrent group had NMIBC with 85% underwent intra vesical BCG therapy. 70% of recurrent group had NMIBC and 43%of them had a history of BCG therapy. They did not find any significant difference in alpha diversity between 2 groups, but beta diversity differed significantly. They stated that Veillonela and Bifidobacterium were more prevalent in recurrent group, whereas Escherchia – Shigella and Heococcus were higher in non-recurrent group.

Pederzoli et al.(32) evaluated the fecal microbiota in patients who underwent neo adjuvant immunotherapy for MIBC. They showed that alpha diversity was higher in patients who responded to pre cystectomy Pembrolizumab, but beta diversity did not differ between responders and non-responders. Sutterella was enriched in responder group, and Ruminococcus bromii was prevalent in non-responders.

Sweis et al.(33) assessed urinary microbiota in 31 patients with NMIBC who received intravesical BCG therapy. They showed that Proteobacteria was the most prevalent phylum, and Enterobacteriales were the most abundant order in the recurrent group, while Lactobacillales were higher in non-recurrent group.

DISCUSSION

The current systematic review aimed to assess the potential role of the urinary microbiota in the development of BC. All in all, the included studies indicate that the composition of urinary microbiota is altered in patients with BC. However, the differentially enriched genera in the urine of patients with BC varied across different studies. While spatio-temporal differences may have contributed to these heterogeneities in urinary microbiota, it is clear that a landscape map of microbiota distribution is necessary. The urinary microbiota is not a one-to-one reflection of the cancerous tissue microbiota, which, in turn, is more likely to be involved in the pathogenesis of BC. Moreover, the urinary microbiota is highly dependent on the type of sampling method. Taken together, therefore, based on the current studies one cannot identify the urinary microbiome as a source of information regarding bladder carcinogenesis(11).

Despite ongoing endeavors, the exact underlying molecular carcinogenesis of BC has not yet been understood(34-36). BC is a commonly diagnosed malignancy worldwide and places a considerable burden on societies due to its significant morbidity and mortality (37-39). Even though the incidence of BC is higher in males than females, women have a worse survival to thoroughly elucidate sex-mediated differences in the composition of urinary microbiota, it would be better to study both male and female patients rather than simply focus on males(40,41).

Future studies are necessary to optimize current methodological approaches to evaluating the impact of dysbiosis on bladder carcinogenesis, tumor invasiveness, progression, and metastasis.

Several limitations exist to our study. It included only a limited number of studies that met our inclusion criteria. In the included studies, some did not have healthy controls and some other biases exist within each of the studies in this emerging area. Mainly the sampling methods differed between the included studies and are still not standardized. Well-designed prospective studies are needed to elucidate the possible role of urine microbiota across the natural history of BC and in its response to therapy.

CONCLUSIONS

The included studies indicate that the composition of urinary microbiota is altered in patients with BC, but no clear conclusions can be made. The sample collection method is highly heterogeneous requiring standardization. The significantly enriched suspicious genera in the urine of patients with BC varied between the different studies. There is no doubt that further research is necessary to uncover the role of microbiota in BC carcinogenesis, invasiveness, progression, and metastasis as well as a predictor/monitor to therapy.

SUMMARY

This review of 27 studies involving 926 individuals found that urinary microbiota differed in bladder cancer patients compared to controls. However, variations in enriched microbial genera and sampling methods require standardization. Further research is needed to clarify the role of microbiota in BC.

ACKNOWLEDGEMENTS

We express our gratitude to the Vice Chancellor for Research and Technology for approval to the protocol.

CONFLICT OF INTEREST

None declared by the authors.

APPENDIX

https://journals.sbmu.ac.ir/urolj/index.php/uj/libraryFiles/downloadPublic/61

REFERENCES

1. Weinstock GM. Genomic approaches to studying the human microbiota. Nature. 2012;489:250-6.
2. Aragón IM, Herrera-Imbroda B, Queipo-Ortuño MI, et al. The Urinary Tract Microbiome in Health and Disease. Eur Urol Focus. 2018;4:128-38.
3. Brubaker L, Wolfe AJ. The female urinary microbiota, urinary health and common urinary disorders. Ann Transl Med. 2017;5:34.
4. Antunes-Lopes T, Vale L, Coelho AM, et al. The Role of Urinary Microbiota in Lower Urinary Tract Dysfunction: A Systematic Review. Eur Urol Focus. 2020;6:361-9.
5. Bersanelli M, Santoni M, Ticinesi A, Buti S. The Urinary Microbiome and Anticancer Immunotherapy: The Potentially Hidden Role of Unculturable Microbes. Target Oncol. 2019;14:247-52.
6. Sung H, Ferlay J, Siegel RL. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. 2021;71:209-49.
7. Rozanec JJ, Secin FP. [Epidemiology, etiology and prevention of bladder cancer.]. Arch Esp Urol. 2020;73:872-8.
8. Mancini M, Righetto M. Spotlight on gender-specific disparities in bladder cancer. 2020;87:103-14.
9. Bi H, Tian Y, Song C, et al. Urinary microbiota - a potential biomarker and therapeutic target for bladder cancer. J Med Microbiol. 2019;68:1471-8.
10. Hourigan SK, Zhu W, S.W.Wong W, et al. Studying the urine microbiome in superficial bladder cancer: samples obtained by midstream voiding versus cystoscopy. BMC Urology. 2020;20:5.
11. Mansour B, Monyók Á, Makra N, et al. Bladder cancer-related microbiota: examining differences in urine and tissue samples. Scientific Reports. 2020;10:11042.
12. Chipollini J, Wright JR, Nwanosike H, et al. Characterization of urinary microbiome in patients with bladder cancer: Results from a single-institution, feasibility study. Urologic Oncology: Seminars and Original Investigations. 2020;38:615-21.
13. Zeng J, Zhang G, Chen C, et al. Alterations in Urobiome in Patients With Bladder Cancer and Implications for Clinical Outcome: A Single-Institution Study. Front Cell Infect Microbiol. 2020;10:555508.
14. Hussein AA, Elsayed AS, Durrani M, et al. Investigating the association between the urinary microbiome and bladder cancer: An exploratory study. Urol Oncol. 2021;39:370.e9-.e19.
15. Pederzoli F, Ferrarese R, Amato V, et al. Sex-specific Alterations in the Urinary and Tissue Microbiome in Therapy-naïve Urothelial Bladder Cancer Patients. Eur Urol Oncol. 2020;3:784-8.
16. Bučević Popović V, Šitum M, Chow CT, Chan LS, Roje B, Terzić J. The urinary microbiome associated with bladder cancer. Sci Rep. 2018;8:12157.
17. Wu P, Zhang G, Zhao J, et al. Profiling the Urinary Microbiota in Male Patients With Bladder Cancer in China. Front Cell Infect

Microbiol. 2018;8:167.

18. Oresta B, Braga D, Lazzeri M, et al. The Microbiome of Catheter Collected Urine in Males with Bladder Cancer According to Disease Stage. J Urol. 2021;205:86-93.

19. Mansour B, Monyók Á, Gajdács M, et al. Bladder tissue microbiome composition in patients of bladder cancer or benign prostatic hyperplasia and related human beta defensin levels. Biomedicines. 2022;10:1758.

20. Hrbáček J, Tláskal V, Čermák P, Hanáček V, Zachoval R. Bladder cancer is associated with decreased urinary microbiota diversity and alterations in microbial community composition; 2023.

21. Qin C, Chen Z, Cao R, Shi M, Tian Y. Integrated Analysis of the Fecal Metagenome and Metabolome in Bladder Cancer in a Chinese Population. Genes. 2022;13:1967.

22. Parra-Grande M, Oré-Arce M, Martínez-Priego L, et al. Profiling the bladder microbiota in patients with bladder cancer. Frontiers in Microbiology. 2022;12:718776.

23. Liu F, Liu A, Lu X, et al. Dysbiosis signatures of the microbial profile in tissue from bladder cancer. Cancer Med. 2019;8:6904-14.

24. Ozer MS, Yildiz HA, Incir C, et al. Urinary microbiota; Which non-ınvasive urine collection method should we use? International Journal of Clinical Practice. 2021;75:e14193.

25. Mai G, Chen L, Li R, Liu Q, Zhang H, Ma Y. Common core bacterial biomarkers of bladder cancer based on multiple datasets. BioMed research international. 2019;2019:4824909.

26. Zhang Y, Wang W, Zhou H, Cui Y. Urinary Eubacterium sp. CAG: 581 Promotes Non-Muscle Invasive Bladder Cancer (NMIBC) Development through the ECM1/MMP9 Pathway. Cancers. 2023;15:809.

27. Sun J-X, Xia Q-D, Zhong X-Y, Liu Z, Wang S-G. The bladder microbiome of NMIBC and MIBC patients revealed by 2bRAD-M. Frontiers in Cellular and Infection Microbiology. 2023;13:1182322.

28. Qiu Y, Gao Y, Chen C, et al. Deciphering the influence of urinary microbiota on FoxP3+ regulatory T cell infiltration and prognosis in Chinese patients with non-muscle-invasive bladder cancer. Human Cell. 2022;35:1-11.

29. Bukavina L, Calaway AC, Isali I, et al. Characterization and functional analysis of microbiome in bladder cancer: American Society of Clinical Oncology; 2022.

30. Knorr J, Werneburg G, Adler A, et al. Pd12-01 bladder tumor microbiome may augment response to BCG in non-muscle invasive bladder cancer. The Journal of Urology. 2022;207:e195.

31. Houenstein H, Ramahi Y, Shah AA, et al. MP59-07 DOES THE URINARY MICROBIOME PROFILE CHANGE AFTER TREATMENT OF BLADDER CANCER? The Journal of Urology. 2022;207:e1003.

32. Pederzoli F, Locatelli I, Riba M, et al. PD42-05 FECAL MICROBIOTA PROFILING OF PATIENTS TREATED WITH NEOADJUVANT PEMBROLIZUMAB FOR MUSCLE INVASIVE UROTHELIAL CARCINOMA. The Journal of Urology. 2021;206:e726-e.

33. Sweis* R, Golan S, Barashi N, et al. MP43-11 CHARACTERIZATION OF THE URINARY MICROBIOME IN PATIENTS WITH NON-MUSCLE INVASIVE BLADDER CANCER TREATED WITH BACILLUS CALMETTE-GUéRIN IMMUNOTHERAPY. The Journal of Urology. 2019;201:e621-e.

34. Antoni S, Ferlay J, Soerjomataram I, Znaor A, Jemal A, Bray F. Bladder Cancer Incidence and Mortality: A Global Overview and Recent Trends. Eur Urol. 2017;71:96-108.

35. Kluth LA, Black PC, Bochner BH, et al. Prognostic and Prediction Tools in Bladder Cancer: A Comprehensive Review of the Literature. Eur Urol. 2015;68:238-53.

36. Tilki D, Burger M, Dalbagni G, et al. Urine markers for detection and surveillance of non-muscle-invasive bladder cancer. Eur Urol. 2011;60:484-92.

37. Putluri N, Shojaie A, Vasu VT, et al. Metabolomic profiling reveals potential markers and bioprocesses altered in bladder cancer progression. Cancer Res. 2011;71:7376-86.

38. Gontero P, Sylvester R, Pisano F, et al. Prognostic factors and risk groups in T1G3 non-muscle-invasive bladder cancer patients initially treated with Bacillus Calmette-Guérin: results of a retrospective multicenter study of 2451 patients. Eur Urol. 2015;67:74-82.

39. Margulis V, Shariat SF, Ashfaq R, Sagalowsky AI, Lotan Y. Ki-67 is an independent predictor of bladder cancer outcome in patients treated with radical cystectomy for organ-confined disease. Clin Cancer Res. 2006;12:7369-73.

40. Kluth LA, Rieken M, Xylinas E, et al. Gender-specific differences in clinicopathologic outcomes following radical cystectomy: an international multi-institutional study of more than 8000 patients. Eur Urol. 2014;66:913-9.

41. Mir C, Shariat SF, van der Kwast TH, et al. Loss of androgen receptor expression is not associated with pathological stage, grade, gender or outcome in bladder cancer: a large multi-institutional study. BJU Int. 2011;108:24-30.