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Bladder Microbiota Snapshots Help to Monitor Urinary Tract Infections in Vulnerable Patients

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To the editor.

Microbiota analysis using next-generation sequencing is undoubtedly a valuable tool in the pipeline of microbiologic investigations. However, to date, nobody can define a standardized healthy microbial community. Moreover, microbiota analysis is not included in any diagnostic guidelines.[1] The urinary microbiota plays a crucial role in maintaining human homeostasis. Normal flora competes with pathogenic species for energy sources, produces antimicrobial molecules, and contributes to urothelial integrity.[2,3] Most of the studies published on urinary microbiota merely provide "snapshots" of microbiota associated with a disease as they lack a comparison to the gold standard urine culture, which is the gold standard for diagnosing urinary infections and asymptomatic bacteriuria. However, in specific at-risk populations, it may fail to produce a positive result and delay the administration of targeted treatment.

The use of metagenomics could identify the presence of pathogenic species and resistance genes in the urinary tract of at-risk individuals and guide subsequent antibiotic therapy, reducing unnecessary exposure to broad-spectrum antibiotics.[4] Although “snapshots” may contribute to elucidating factors that perturb the microbiota and what impact these changes have on the development of disease, the evaluation of longitudinal data may be useful in establishing the significance of these changes.

In this context, several investigations have failed to address the association between urinary microbiota and long-term patient outcomes.[5] Spinal dysraphism (also known as spina bifida) causes neurological deficits, including neurogenic bladder, resulting in detrusor sphincter dyssynergia. This condition represents a predisposing factor for urinary tract infections (UTIs).[6,7]

In this work, we routinely followed up a pediatric cohort with spinal dysraphism for 3 years after microbiota profiling to establish any correlation between this and the development of subsequent UTIs.

We studied microbiota in a cohort that included individuals who were not taking antibiotics or probiotics and did not have a UTI at the time of recruitment.[8] The relative abundance of neuropathological species in samples from patients with spina bifida increased over time, while patients routinely performing clean intermittent catheterization (CIC) had a predominance of skin organisms. 16s sequencing raw data obtained from 43/44 specimens were analyzed at the genus level, selecting the top 20 genera identified in the dataset. Relative abundances were then matched with Shannon diversity and Pielou’s Evenness (**Figure 1A**). Alpha diversity metrics, as expected, showed a positive Pearson correlation ($p<0.001$, Rho=0.924), suggesting that with the decrease in species number, one or more microbes prevailed in the community. Low values of both alpha diversity metrics were associated with a higher relative abundance of genera manifestly recognized as uropathogens.

We followed up on these patients for 3 years and carried out a relationship between urine culture results and the most abundant genera. 39/44 individuals had a urine culture examination. 18 samples were positive at the first collection (median = 12 months); 10/28 positive samples at the second time point (median = 11 months), 2 of whom belonged to previous negative samples; 5/15 positive samples at the third urine collection (median = 11 months), one of whom belonged to previous negative patients (**Figure 1B**).

Interestingly, 12/43 (28%) samples showed *E. coli-Shigella* as the most abundant genus, 8 of whom (67%) were associated with culture-positive samples at the first follow-up (6 samples for gram-negative bacteria and 2 samples for both gram-negative and gram-positive bacteria). While *the Burkholderia* genus was always associated with a positive urine sample, *Bacteroides*, *Cutibacterium*, *Prevotella*, and *Subdoligranulum* genera

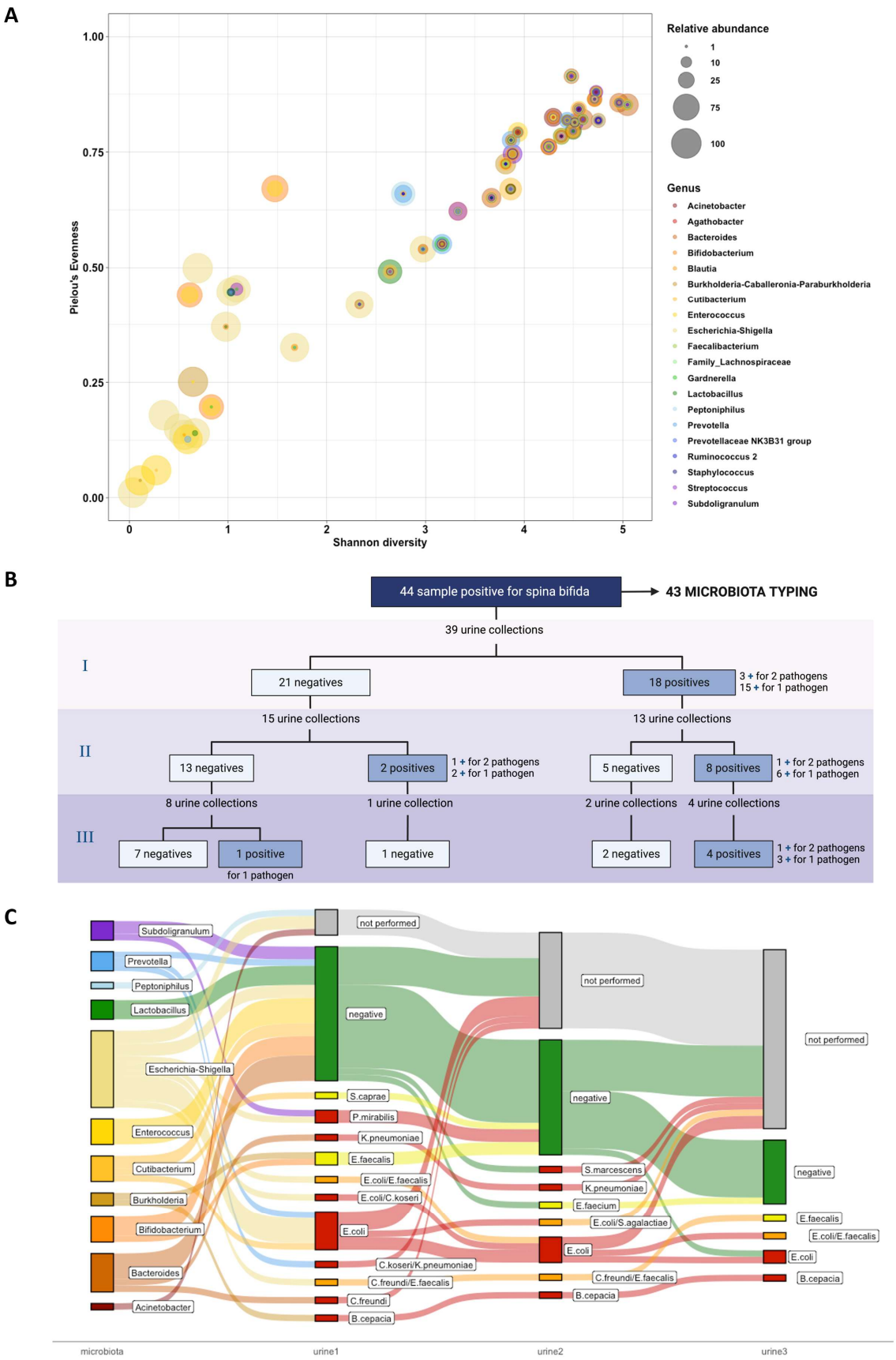

Figure 1: A. Bubble chart reporting Shannon diversity index and Pielou's Evenness. The bubble's size shows the relative abundances of the top 20 genera identified with metagenomic assessment. **B.** Schematic representation of the experimental setting. Each horizontal box shows the I, II and III urine collection following microbiota characterization. The result of each urine culture is reported. **C.** Sankey diagram highlighting the association between the top genus for each sample and the microbiological result obtained by urine cultures at I, II and III collections after microbiota evaluation. Gray boxes show not performed urine cultures, whereas green ones show negative results. Positive urine cultures are evidenced as red box (gram-negative bacteria), yellow box (gram-positive bacteria) and orange boxes (gram-negative and gram-positive bacteria).

were only partially associated with a positive sample at the first follow-up. Interestingly, samples dominated by *Enterococcus*, *Lactobacillus*, and *Bifidobacterium* genera appeared mostly associated with negative urine samples (**Figure 1C**). These findings suggested that the prevalence of specific genera may have a protective activity against UTIs, leading to even reevaluating the role of some bacteria. *Enterococcus* and *Lactobacillus* are two of the most renowned genera that produce antimicrobial peptides (such as bacteriocins).[9] Of note, 15/18 of urine positive at the first follow-up belonged to patients managed with CIC, highlighting this as a major risk factor for the development of UTI. This result is opposite to what was shown by *Kaye et al.*, suggesting a further deep clinical investigation in these patients.[6]

Although it is plausible that the small sample size might have influenced our results (including the sample drop-out during follow-up), our findings prompt 16s analysis as a promising candidate to investigate urinary tract infections in special at-risk populations as patients with neurogenic bladder, vesicoureteral reflux, renal abnormalities or a history of recurrent UTIs. While urine culture remains the gold standard for the diagnosis of urinary tract infections, the characterization of bladder microbiota may represent an innovative and additional diagnostic tool for the diagnosis of UTIs in vulnerable populations.[10,11]

On the other hand, further longitudinal studies will be needed to harmonize microbiota characterization with clinical outcomes, avoid the use of inappropriate antibiotic prophylaxis, and opportunely set conventional and non-conventional antimicrobial interventions.

Ethics approval and consent to participate. The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Policlinico Universitario "A. Gemelli" IRCCS for studies involving humans (ID: 4279). Written informed consent was obtained from each subject involved in the study.

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Authors' contributions. This study was designed by F.D.M and C.R. Data collection and analysis by F.D.M. and G.S. Figure preparation was conducted by F.D.M. and G.S. F.D.M., G.S. and D.M.B. wrote the initial draft. M.S. and C.R. revised the final draft. All authors participate in the discussion and interpretation of the results. All authors have read and agreed to the published version of the manuscript.

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