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Introduction & Objectives: Recently, advanced culture-independent and -dependent methodological approaches have demonstrated significant changes in urinary microbiota of women with overactive bladder (OAB). Nevertheless, the aetiology and effective treatment of OAB remain unclear [1-3], and for its diagnosis it is still mandatory to exclude a urinary tract infection (UTI), based on a standard culture protocol, with limited sensitivity and specificity. In this study, we aim to comprehensively investigate the urinary bacterial composition of OAB women, using optimized culturomics (CULT) and community amplicon sequencing (CAS).

Materials & Methods: Following Hospital São João ethical approval, mid-stream urine samples were collected from 6 pre- and postmenopausal women (mean age: 56 years; range: 36-80 years) with OAB symptoms. Symptoms severity was assessed with Overactive Bladder Symptom Score (OABSS) [4]. Exclusion criteria included: Current UTI (based on urinalysis and standard urine culture, SUC), antibiotic exposure in the past 4 weeks, pregnancy, history of pelvic radiotherapy, bladder tumor, urolithiasis and neurogenic voiding dysfunction. To characterize urinary microbiota, we used optimized CULT [different media and culture conditions, MALDI-TOF/MS and/or genotypic biomarkers for isolate identification]. Concurrently, all samples were subjected to CAS using V1-V8 regions of 16S rRNA by PacBio.

Results: In all patients (mean OABSS: 8; range: 7-9), CULT revealed large numbers of bacteria (10^4 - 10^8 CFU/ml), with identification of 71 species, belonging to 36 genera, although no criteria for UTI were found based on routine urinalysis and SUC. In extensive analysis of each urine sample, we identified: i) a dominance of *Gardnerella vaginalis* (59%; 63% by CULT) and *Lactobacillus iners* (43%; 90% by CAS) in 2 samples; ii) a high amount of *Bifidobacterium* spp. (28% by CULT), *Corynebacterium aurimucosum* (22% by CULT) and diverse uropathogens species; iii) a dominance of *E. coli* together with other potential uropathogens (e.g. *Fingoldia magna*, *Bacteroides vulgatus*); iv) a dominance of *Enterococcus faecalis* in an unbalanced microbiota enriched in *Staphylococcus* species; and v) a high amount of *G. vaginalis* (46% by CULT) and *Ureaplasma urealyticum* (44% by CAS) in a patient with severe symptoms (OABSS: 9).

Conclusions: All OAB patients presented an unbalanced urinary microbiota with a dominance of bacterial species known to be involved in UTI, but not detected by SUC. Our study strongly supports the need to urgently revise routine microbiological methodologies used for OAB diagnosis, as well as to rethink treatment strategies, covering uncommon uropathogens.

References:

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