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Short communication

An *in vitro* model of catheter-associated urinary tract infections to investigate the role of uncommon bacteria on the *Escherichia coli* microbial consortium

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ABSTRACT

Uncommon bacteria, such as *Delftia tusurhatensis* have been isolated from CAUTIs in combination with well-established pathogenic bacteria such as *Escherichia coli*. Nonetheless, the reason why *E. coli* coexists with other bacteria instead of outcompeting and completely eliminating them is unknown. As such, a flow cell reactor simulating the hydrodynamic conditions found in CAUTIs (shear rate of 15 s^{-1}) was used to characterize the microbial physiology of *E. coli* and *D. tsuruhatensis* individually and in consortium, in terms of the growth kinetics and substrate uptake. Single-species biofilms showed that up to 48 h the cultivable cell counts significantly increased for both species ($p < 0.05$). When in dual-species biofilm, *E. coli* outnumbered *D. tsuruhatensis* up to 16 h and then *D. tsuruhatensis* gained a fitness advantage. However, the assessment of the spatial distribution of the dual-species biofilm by LNA/2′OMe-FISH revealed that *E. coli* and *D. tsuruhatensis* coexist and tend to co-aggregate over time, which suggests that both bacteria are able to cooperate synergistically. Substrate uptake measurements revealed that *D. tsuruhatensis* metabolized citric acid more rapidly, presumably leaving more uric acid available in the medium to be used by *E. coli*. In conclusion, *E. coli* and uncommon bacteria seem to cooperate, when sharing the same environment under dynamic conditions, leading to the persistence of both bacteria in a stable microbial community.

1. Introduction

In hospitals and nursing homes, there is a regular occurrence of infections, of which about 9% are attributed to catheter-associated urinary tract infections (CAUTIs) [1]. Urinary catheters are medical devices used in patients to control the urine drain due to incontinence problems or post-operative urine retention [2]. Unfortunately, most patients experience long-term catheterization, which is frequently associated with polymicrobial infections [3–6]. In fact, with time, microorganisms end up forming polymicrobial biofilms on the surface of urinary catheters [7–9].

A biofilm is a community of microorganisms adhered to a biotic or abiotic surface, which is enclosed in an extracellular polymeric substances (EPSs) matrix. Compared to planktonic microorganisms, they have an altered phenotype associated with a reduced growth rate, a high tolerance to antimicrobial agents and to the host immune system, and an altered expression of specific genes [10–13]. Typically, microbial biofilms display a coordinated and cooperative behavior [14], where the concentration of individual populations is adjusted according to the conditions found in the environment [15].

Studies that combine *E. coli* with other pathogenic bacteria, have already been addressed in context of CAUTIs (e.g. [25]). Recently, uncommon bacteria such as *Delftia tusurhatensis* have been isolated and identified in nosocomial infections involving polymicrobial

Abbreviations: EPS, extracellular polymeric substance; FISH, fluorescence *in situ* hybridization; CLSM, confocal laser scanning microscopy; LNA, locked nucleic acid; 2′OMe, 2′-O-Methyl-RNA; CAUTIs, catheter-associated urinary tract infections; TSA, tryptic soy agar; CFUs, colony forming units; O.D., optical density; AUM, artificial urine medium; Υ, shear strain rate; τ_w, shear stress; μ, fluid viscosity; Q, flow rate; Re, Reynolds number; ρ, fluid density; W, fitness value; m, Malthusian parameter.

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biofilms [7,16], including CAUTIs [7]. The pathogenic potential of these uncommon bacteria is undefined. Nonetheless, this type of bacteria appears in catheter-associated biofilms in combination with well-established pathogenic bacteria (e.g. *Escherichia. coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*) [7]. The role of uncommon bacteria is underestimated probably due to the absence of commercial media and kits to detect these bacteria in hospitals. But, as reported in previous studies [15,17], *E. coli* and uncommon bacteria are good biofilm producers on abiotic surfaces (e.g. silicone, polystyrene), and when in co-culture they are able to form a stable microbial consortia, where both bacteria coexist, even when inoculated at different proportions. Also, the analysis of ecological interactions between *E. coli* and uncommon bacteria has revealed that these bacteria tend to interact either synergistically or, at least, display a neutralism behavior. These previous studies have only described the possible synergistic interactions assessing bacterial adhesion, biofilm formation and overall antibiotic resistance of mixed-species consortia in static conditions. Nonetheless, knowledge about the microbial physiology of *E. coli* and *D. tsuruhatensis* under dynamic conditions, especially when concerning nutritional requirements, remains unknown. Hence, a pressing need exists for research directed toward understanding microbial interactions that drive CAUTIs biofilm communities involving uncommon and pathogenic bacteria under dynamic conditions.

A flow cell system simulating the shear strain rate found in urinary catheters ($15\ s^{-1}$) [18–20] was used and species behavior was then evaluated individually and in consortium, when exposed to artificial urine medium (AUM) flow and to the silicone material. The physiology of each bacterium was characterized in terms of the growth kinetics and the substrate's uptake (lactic acid, urea, citric acid, creatinine and uric acid) under dynamic conditions.

2. Material and methods

2.1. Strains and culture media

E. coli CECT 434 and *D. tsuruhatensis* BM90 were recovered from a frozen stock (–80 °C), streaked on Tryptic Soy Agar (TSA) (Merk, Germany) and grown overnight at 37 °C. For the inocula preparation, each bacterium was inoculated in 250 ml of AUM and cultures were placed in an incubator (AGITORB 200, Aralab, Portugal) during 16–18 h at 37 °C and 150 rpm. AUM was prepared as previously described [21], but as yeast extract and peptone are a mixture of polypeptides and amino acids, they were not added to the medium to enable the measurement of substrate's uptake. Then, cell concentration was assessed by optical density (O.D.) at 620 nm and the inocula were diluted in AUM in order to obtain a final concentration of 10^8 CFU.ml^{-1}. Each diluted inoculum was used to inoculate the reactor system during 1 h at a flow rate (Q) of 0.5 mlmin^{-1}.

2.2. Determination of bacterial growth rates

The growth rate for each bacterium was determined in AUM (without yeast extract and peptone) in a batch culture as described in a study performed by Azevedo et al. [17].

2.3. Flow cell reactor setup

The reactor system used in this study (Fig. 1) consists of a vertical flow cell, water bath at 37 °C, peristaltic (B1) and centrifugal pumps (B2), vessel containing the nutrients, recirculating silicone tubes and a waste vessel. The flow cell used is a rectangular Perspex column with 10 apertures in removable rectangular pieces of Perspex where silicone coupons were placed. All specifications of the flow cell reactor and on the assembling of the system are provided in Supplementary material (Supplementary Method 1).

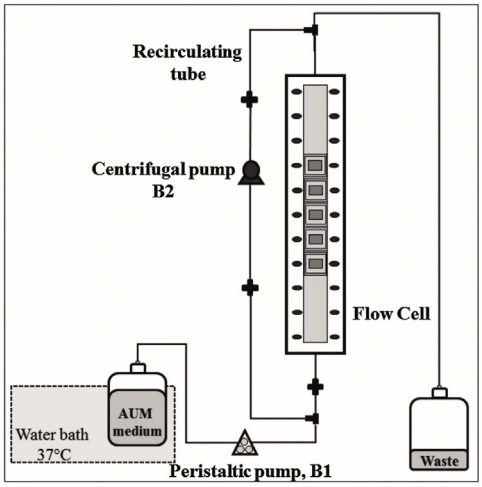

Fig. 1. Schematic representation of the flow cell reactor. Pumps B1 and B2 controlled the flow rate of AUM (0.5 ml min^{-1}) and the velocity of recirculating fluid (300 ml min^{-1}), respectively.

While the dynamic system used here has geometric features distinct from urinary catheters, important aspects involved in CAUTIs were simulated such as the composition of the AUM, which was reported as suitable to mimic the urine in a wide range of microbial studies [21–25]. The temperature of AUM was kept at 37 °C, corresponding to the human body temperature. In addition, the flow rate was adjusted to mimic a natural urine flow (see calculations in Supplementary Method 1); it should be noticed that within the human body the catheter will be subjected to an intermittent flow, with periods from absent to high flow rates. As previous experiments were performed under static conditions [15,17], the dynamic system used in present study allowed to simulate the periods of high urine flow rate. Also, this system only allows a correct simulation of an intraluminal colonization, as flow is restricted to the catheter lumen.

After defining the system settings, biofilms were formed and analyzed as described in Supplementary Method 1. The fitness values (W) and Malthusian parameters (m) were determined as previously described [15,17].

2.4. Statistical analysis

For each parameter, the average and standard deviation were calculated. Results were compared using ANOVA by applying Levene's test of homogeneity of variance and the Tukey multiple-comparisons test, using the SPSS software. Statistical tests were carried out at a significance level of 0.05.

3. Results and discussion

Microbial infections in catheterized patients are usually composed by a dominant pathogenic bacterium (e.g. *E. coli*) which might interact and coexist with other pathogens (e.g. *P. aeruginosa*, *E. faecalis* and *P. mirabilis*) [9,26,27], or even, with uncommon bacteria with a poorly understood role such as *D. tsuruhatensis* [7]. Hence, a polymicrobial community is established on the surface of an urinary catheter, in particular when the urinary catheter remains in the patient for several weeks or months [3–6]. Recently, some studies have studied the role of some uncommon bacteria in clinical infections and have highlighted their role in shaping the overall behavior of the microbial biofilm [15,17,28,29].

In present work, the physiology of *E. coli* and the uncommon bacteria *D. tsuruhatensis* under dynamic conditions similar to those found in catheterized urinary tract was evaluated, both in terms of