



# Assessing composition and micromorphology of biofilm in urinary catheters



I Caola<sup>1</sup>, F Tessarolo<sup>2,3</sup>, M Leonardi<sup>1</sup>, T Cai<sup>1</sup>, F Piccoli<sup>1</sup>, G Nollo<sup>2,3</sup>, G Malossini<sup>1</sup>, R Bartoletti<sup>4</sup>, P Caciagli<sup>1</sup>

<sup>1</sup>APSS, Trento, Italy; <sup>2</sup>University of Trento, Trento, Italy; <sup>3</sup>FBK, Trento Italy; <sup>4</sup>University of Florence, Florence, Italy

## Introduction

In the last years, the role of biofilm producer microorganisms in catheter-associated urinary tract infections (CAUTIs) has drawn increasing attention. Morphology, strain identification and composition of biofilm on urinary catheter (UC) can contribute to implement both preventative and therapeutic approaches. This study aimed at characterizing microbial colonization and investigating microscopic features of biofilm formed on UC removed from patients.

## Materials and Methods

54 UCs (27 silicon, 20 latex, 7 silver coated; insertion time: 15(3-30) days) from 54 patients (male/female: 49/5; age 70(38-87) years; 89% with antibiotic prophylaxis) were included.

At removal, 1cm segment of the tip was aseptically cut and collected in a container with saline.

Biofilm was sampled by brushing the lumen with a small-size flocked swab, eluting the dislodged material in 50µl of saline and inoculating 1µl in 5% sheep blood agar. Adapted from [1, 2].

An equivalent segment was sonicated in 10mL of trypticase soy broth and 10 µL of sonicated fluid were plated on 5% sheep blood agar [3-4]. Colonies were enumerated and CFU/cm were calculated for the two cultural methods. Cultures were also performed on urine aliquots sampled before UC removal. Biofilm forming ability of isolates was determined by MTT test according to [5].

A third segment was fixed in 70% ethanol, longitudinally sectioned, dehydrated in ascending hydro-alcoholic solutions, air dried, gold sputtered and imaged in a scanning electron microscope (SEM). Crystals composition was assessed by X-ray energy dispersive spectroscopy (EDX).

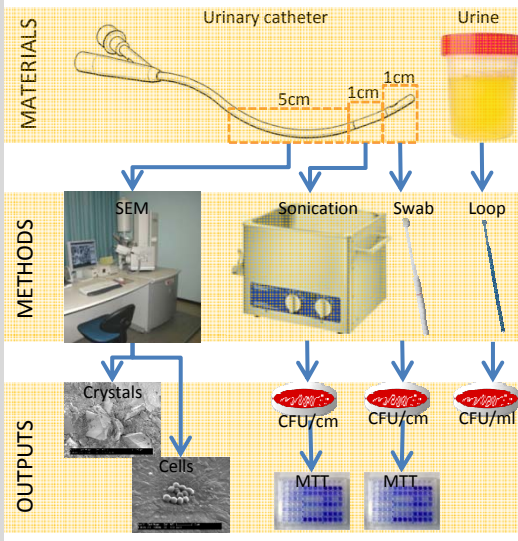


Fig. 1: Experimental flow chart

## Results

A total of 57 strains were isolated (54 from swabbed materials, 47 from sonication fluid). 61% of the UCs showed microbial growth (51% polymicrobial). All UCs inserted for >30 days gave positive cultures (Fig 2). Urine culture allowed the isolation of 60% and 75% of the strains obtained from swabs and sonication respectively. E. coli, E. faecalis, E. faecium were most frequently isolated (>10<sup>5</sup> CFU/cm) with both cultural techniques and were generally non biofilm or weak biofilm producers in vitro (Fig. 3). S. epidermidis strains were in part non producers and in part strong producer strains.

SEM analysis showed the presence of microorganisms organized in biofilm in 53% of UCs with a general agreement between SEM morphotypes and cultural results (Fig. 4a,b,c). Occasionally, SEM analysis showed multiple morphotypes on UCs with single isolate cultures. Crystals adhering to UCs were often embedded in biofilm and atypical crystal morphologies were identified by integrating SEM and EDX information (Fig.4d,e,f).

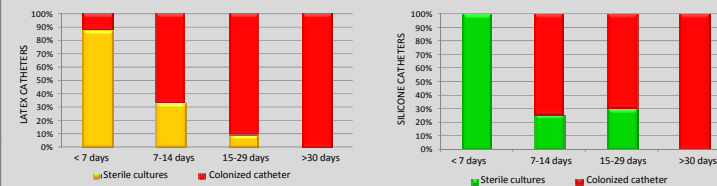


Fig. 2: Colonized catheters vs time. Latex (left), silicone (right).

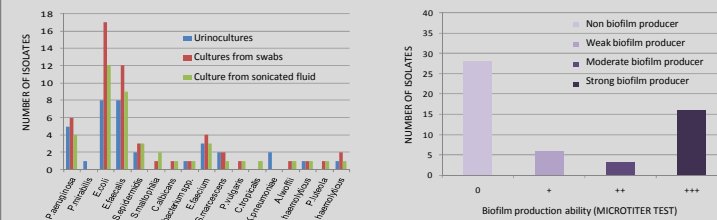


Fig. 3: Isolated strains (left) and their biofilm production ability (right).

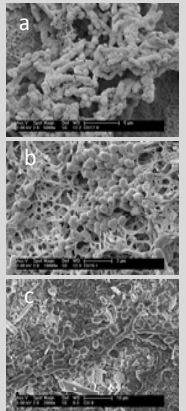
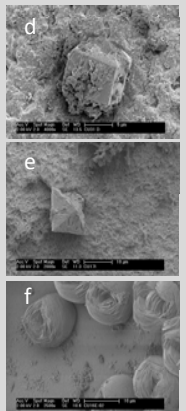


Fig. 4: Biofilm on UCs surface: bacteria (a, b), yeast (c), crystals (d, e, f).



## References

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## Conclusions

Since urine culture is only partly representative of the biofilm forming microorganism in UCs, specific cultural methods of the catheter tip and microstructural analysis of the biofilm should be considered in the characterization of UC biofilm and can give a further insight in the mechanism of crystalline biofilm formation in patients in order to develop new strategies for reducing catheter colonization and CAUTIs.