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Note

A method to assess influence of different medical tubing on biofilm formation by *Acinetobacter baumannii*

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ABSTRACT

In this study, we developed a method to assess influence of different medical tubing on biofilm formation by *A. baumannii*. The results of biofilm quantification and scanning electron microscopy showed that the biofilm formation susceptibility of different tubing materials was rubber latex > polyvinyl chloride > silicone.

Acinetobacter baumannii, an important emerging pathogen that can cause nosocomial infections, is infamous for its ability to form biofilms (Longo et al., 2014). Catheter-related urinary tract infections are mostly caused by biofilm-forming strains of *A. baumannii* (Pour et al., 2011). The *A. baumannii* cells from a biofilm produce higher mortality rates than an equivalent number of planktonic cells (Wand et al., 2012). Bacterial biofilms are proven to be a common cause of persistent infection (Costerton et al., 1999). Indwelling medical devices have been demonstrated to serve as a platform for biofilm formation by *A. baumannii* (Donlan, 2001). The methods of measuring biofilm formation are divided into two categories, direct counting and indirect measurement methods such as XTT assay (Wilson et al., 2017). For example, the XTT assay and microscopy have been used to investigate *Candida* biofilms grown on the polystyrene plate and silicon elastomer surface to compare the influence on biofilm formation between artificial urine and RPMI medium (Uppuluri et al., 2009). However, the study did not analyse the change of biofilm formation on different indwelling medical tubing. Therefore, we proposed a method, in which cut medical tubes were inserted into agar for biofilm formation and viable cells in the biofilm formed on the tubing lumens were quantified.

The clinical isolate *A. baumannii* VGH2 reported in our previous study was used in this biofilm study (Lin et al., 2017). To understand the influence of different medical tubing on biofilm formation by *A. baumannii*, we compared biofilm formation on polyvinyl chloride (PVC) endotracheal tube, silicon and rubber latex Foley catheters.

The PVC endotracheal tube and two kinds of Foley catheters were cut to 1 cm length, sterilized with alcohol and UV light and fixed into a 6-well plate with 1% agar (Fig. 1A). Then, the lumens of the cut tubes were filled with Mueller Hinton Broth (MHB) (Sigma-Aldrich, St. Louis, MO) without and with 0.5 OD bacterial cells, after the agar had solidified and incubated for 24, 48, or 96 h at 37 °C (Fig. 1B). Then the cut tubes were transferred to a 24-well plate, washed with phosphate-buffered saline (PBS), shaken for 5 min at 100 rpm, immersed and incubated in 1 mL of XTT solution (0.25 mg/mL XTT, 0.5 mM menadione in PBS) for 2 h at 37 °C (Fig. 1C). After incubation, 200 μL of the XTT supernatant was transferred to a fresh 96-well plate, and the colourimetric changes were measured at 490 nm. The agar length in the lumen of each tube (agar height) was also measured (Fig. 1D). The determination of biofilm formation was performed by XTT assay with the calculation formula ($OD_{XTT\ cells}$-$OD_{XTT\ control}$)/(diameter*3.14*(1-agar height)) (Chaieb et al., 2011).

For scanning electron microscopy (SEM) examination, the PVC endotracheal tube and two kinds of Foley catheters were cut to the same size circles, which were further fixed on polystyrene coverslips with instant glue and then sterilized with alcohol and UV light. An inoculum of *A. baumannii* cells (OD_{600} 0.01) was suspended in 6-well polystyrene plates with 5 mL of MHB containing the polystyrene coverslip fixed with circles, which was then incubated for 96 h at 37 °C. The samples were fixed in 3.7% formaldehyde for 40 min at room temperature and then rinsed twice with PBS. Finally, the circles cut from the endotracheal tube and Foley catheters on the polystyrene coverslip were examined by SEM as previously described (Djeribi et al., 2012).

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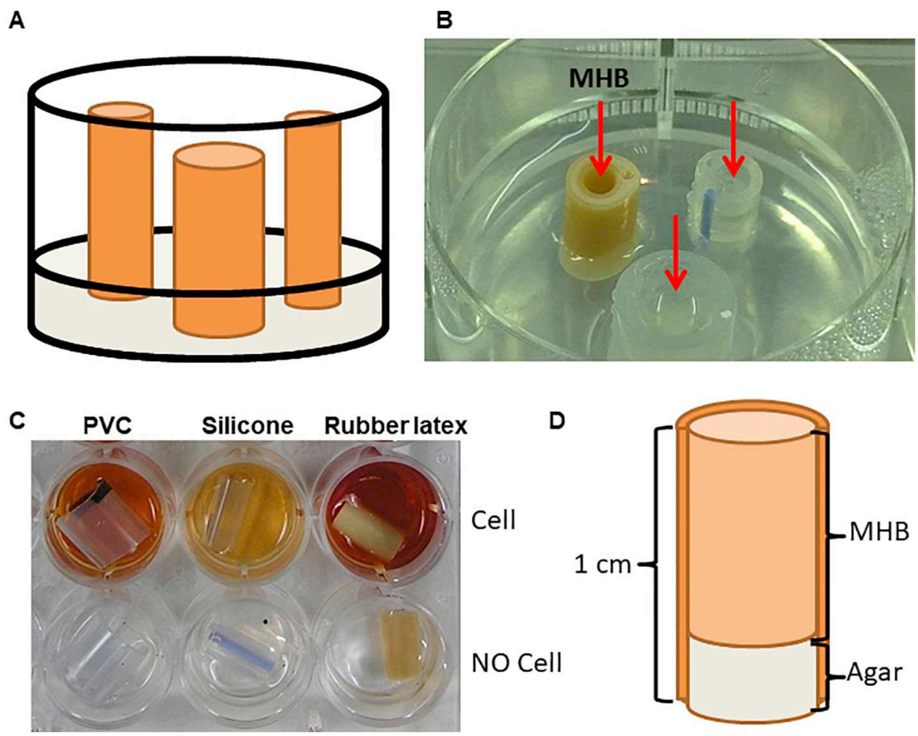

Fig. 1. (A) The drawing and (B) photo of sterilized PVC endotracheal tube and two kinds of Foley catheters fixed into a plate with 1% agar. The lumens of the cut tubes were filled with MHB (red arrows) after the agar had solidified. (C) Reaction of biofilm and XTT solution. (D) A longitudinal section of the cut tube diagram to show the agar length in the lumen (agar height). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

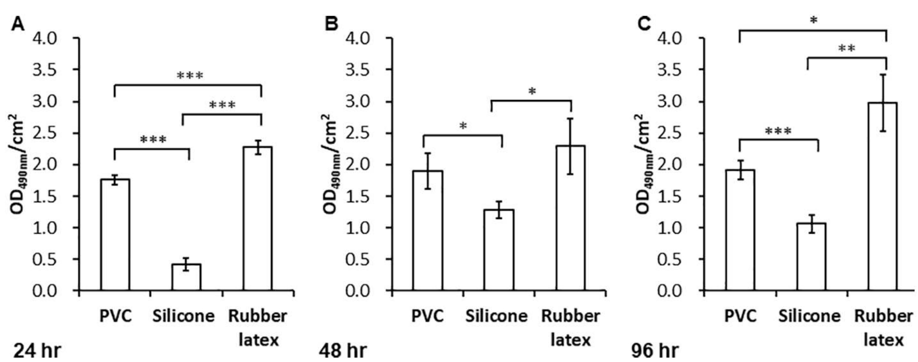

Fig. 2. Influence of PVC, silicon and rubber latex as a medium for biofilm formation by *A. baumannii*. Determination of biofilm formation on the lumens of the cut tubes after incubation for (A) 24, (B) 48 or (C) 96 h.

The comparison of biofilm formation by *A. baumannii* VGH2 on PVC, silicone and rubber latex is shown in Fig. 2. Of the three tested cut tubes, rubber latex was at the top of the ranking in biofilm formation susceptibility, followed by PVC and silicone with statistical significance, whether it was incubated for 24 h, 48 h or 96 h.

The SEM images of biofilm formation on silicon and rubber latex Foley catheters by *A. baumannii* VGH2 (incubation for 96 h) are shown in Fig. 3. Both biofilm formation and SEM imaging supported the conclusion that the biofilm formation susceptibility of different tubing materials by *A. baumannii* was rubber latex > PVC > silicone.

A. baumannii contains several virulence factors, including extracellular components with haemolytic, phospholipase, protease and iron-chelating activities, biofilm formation, surface motility, and stress resistance (Antunes et al., 2011). Of all these virulence factors, biofilm-forming ability is considered a major factor common to a large number of *A. baumannii* clinical isolates (Longo et al., 2014). To better understand and control biofilms on indwelling medical tubing of different materials, reliable sampling and measurement techniques must be developed to investigate the role of biofilm formation in antimicrobial drug resistance and infection control. Although there are many different methods to assess biofilm growth, many of them are inconvenient because they are expensive, time-consuming, requiring experienced skills and unable to differentiate between live and dead cells (Wilson et al., 2017). Besides, few studies are directly determining biofilm formation on the lumen of medical tubing in vitro. In this study, we established a practical agar-plate method with XTT assay to compare the biofilm formation by *A. baumannii* on the lumens of different medical tubes in vitro, which can be used in the future studies. Furthermore, this study showed that the biofilm formation susceptibility ranking of different materials was rubber latex, PVC and silicone, so we urge against the use of latex rubber as the material for urinary catheters in clinical practice.

Conflict of interest statement

None declared.

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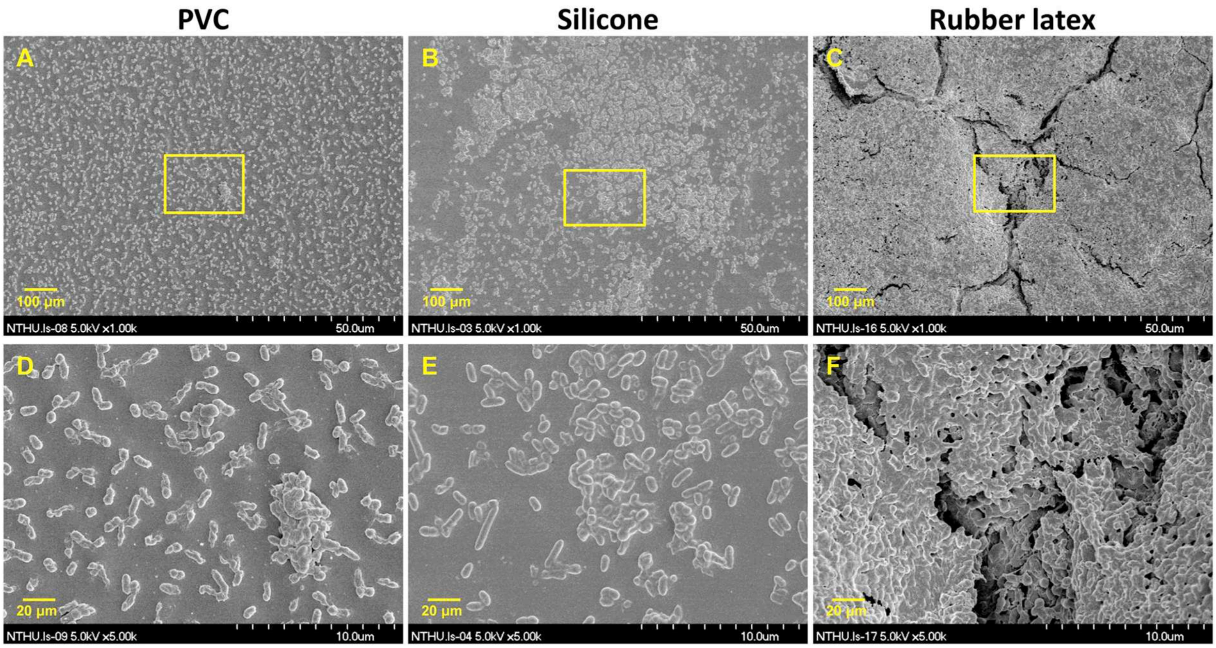

Fig. 3. SEM imaging of biofilm formation by *A. baumannii* on PVC, silicon and rubber latex. (A), (B) and (C) are the images after 96 h of incubation with *A. baumannii* VGH2, whereas (D) (E) and (F) are the zoom images in five times.

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