Biocide activity against urinary catheter pathogens (51 characters with spaces-not to exceed 54 characters)

Biocides against urinary pathogens (31 characters)

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Keywords: *Proteus mirabilis*, biofilm formation, minimum inhibitory concentration, tea tree oil, triclosan, eugenol

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Abstract

Antimicrobial effects of essential oils against bacteria associated with urinary catheter infection was assessed. Tests were performed on 14 different bacterial species cultured either planktonically or as biofilms. Biofilms were found to be up to 8-fold more tolerant of the test agents. Higher antimicrobial tolerance was also evident in tests conducted in artificial urine. Eugenol exhibited highest antimicrobial effects against both planktonic cells and biofilms when compared with terpinen, tea tree oil and cineole.

(73 words, cannot exceed 75 words)

Foley catheters are frequently used to drain urine from the bladder of patients with urinary incontinence or neurological dysfunction. Whilst providing a valuable function, urinary catheters also provide access for microorganisms to infect the bladder and also undermine the basic host defences of the urinary tract. Importantly, catheter-associated urinary tract infections (CAUTIs) represent the most frequently encountered hospital acquired infection (1). *Proteus mirabilis* is a urease positive bacterium that raises urine pH during infection. This allows struvite and apatite crystal encrustation of the catheter which obstructs urine flow, potentially promoting serious clinical complications. All currently available catheters are vulnerable to this encrustation and there is no single effective preventative strategy (2-4).

Due to the increasing prevalence of antibiotic resistance, interest has arisen in the therapeutic use of alternative medicines in combating infection (5-9). Naturally-occurring biocides are effective in inactivating a wide variety of microorganisms as they often target multiple bacterial sites and as such, are less prone to development of resistance compared with antibiotics (10, 11). One possible approach to prevent catheter encrustation would be to incorporate these biocides into catheter wash-out solutions, into the catheter material itself or use the catheter retention balloon as a reservoir for the delivery of the antimicrobial agent into the catheterised bladder (12-16). Little is currently known about the susceptibility of *Proteus* to natural antimicrobial agents hence the aim of this study was to examine antimicrobial activity of several essential oils, namely tea tree oil, terpinen, cineole, and eugenol, against *P. mirabilis* and other urease producing bacteria involved in CAUTIs. Activity of these agents was tested against both planktonic cells and biofilms, as the latter frequently exhibits enhanced resistance to traditional antimicrobials.

The isolates used in this study are presented in Table 1. The antimicrobial agents tested were cineole, tea tree oil (TTO), terpinen, and eugenol. Overnight cultures of test isolates were prepared in Mueller Hinton Broth (MHB) (17) or artificial urine adjusted to pH 6.1, and containing 1% Tryptone Soya Broth (TSB)(18). Cultures were adjusted to a 0.5 MacFarland standard (approximately 10^8 cells ml^{-1}) and diluted 100-fold in MHB or artificial urine. Serial dilutions of the antimicrobial agents were prepared in MHB or artificial urine supplemented with 0.002% (v/v) Tween 80 (Sigma Aldrich, UK). A 100-µl volume of each dilution of antimicrobial agent was added to an equal volume of microbial suspension, giving antimicrobial concentrations ranging from 0.008 to 8% (v/v). Controls included bacterial suspensions containing no antimicrobial agent and uninoculated culture media. The bacteria and antimicrobial agent were co-incubated aerobically in 96-well microtitre plates for 24 h at 37˚C. Microbial growth was determined by spectrophotometric analysis (620_{nm}). Absorbance readings were standardised against 'microbial-free' antimicrobial agent controls. The minimal inhibitory concentration (MIC) was defined as the lowest concentration of antimicrobial agent which resulted in ≥80% reduction in absorbance compared to the antimicrobial-free controls (19).

A biofilm susceptibility test was also performed using the isolates described in Table 1. Isolates were incubated as described above, but without agitation to allow for biofilm formation. Culture medium was removed and the biofilms were washed with 100-µl phosphate buffered saline (PBS) to remove planktonic cells. Fresh culture medium (100 µl), containing antimicrobial agent at concentrations ranging from 0.008 to 8% (v/v), was added to each well. Controls, as previously described, were also included. Biofilms were incubated in the presence of antimicrobial agent for 24 h without agitation under the conditions described above before the supernatant was removed and the biofilm washed (×1) with PBS. Fresh culture medium (100 µl), which did not contain antimicrobial agent, was added to the biofilms which were disrupted by repeated pipetting. The turbidity (620_{nm}) of the re-suspended biofilm was measured and again after incubation at 37°C for an additional 6 h and 24 h. The relative growth of microorganisms was determined by the change in absorbance and antibiofilm activity recorded as the lowest concentration of agent that demonstrated a ≥80% reduction in absorbance compared to the control. All experiments were performed in triplicate on 3 separate occasions in MHB and artificial urine.

A summary of the results is presented in Table 1. Cineole and TTO had the lowest antimicrobial activity against bacteria grown in MHB and were therefore not tested in artificial urine. Highest antimicrobial activity by the essential oils against planktonic growth in MHB occurred with eugenol and terpinen. However, when tested in artificial urine, the activity of terpinen was noticeably reduced. There was also a higher tolerance of biofilms (up to 8-fold) to most of the essential oils compared with their planktonic equivalents. This increased biofilm resistance was least evident with

eugenol in MHB, but was apparent with eugenol (up to 32-fold) against biofilms in artificial urine.

Urinary catheters provide a convenient means to drain urine from the bladder of patients suffering from urinary incontinence or neurological dysfunction. However, they are also associated with complications, as they provide access for bacteria from a heavily contaminated external skin site to the bladder and kidneys (20). Catheters also undermine the normal filling and emptying of the bladder which flushes out microorganisms that might be contaminating the urethra. Furthermore, a reservoir of residual urine remains in the bladder of catheterised patients, allowing continued proliferation of contaminating organisms (21). CAUTIs are usually asymptomatic, and because of the danger of promoting antibiotic resistance, catheter associated bacteriuria is generally not treated with antibiotics (22-24). Elimination of *P. mirabilis* by appropriate therapy as soon as it enters the catheterised urinary tract would reduce the incidence of CAUTIs and improve the quality of life for many patients, whilst also reducing the costs of managing the complications of catheter encrustation and blockage (25). Several management and treatment strategies for CAUTIs have been used, including limiting catheter use, removal of the catheter as soon as possible, maintaining a closed drainage system, and use of alternative catheter surfaces with anti-infective agents (23, 26). Unfortunately, no single effective strategy for prevention of CAUTIs has yet been identified.

The purpose of this study was to assess the antimicrobial activity of several essential oils against bacteria involved in CAUTIs. Based on these findings, further studies are planned to incorporate these agents into urinary catheter materials to prevent infection. The results of this study show that TTO, terpinen-4-ol, eugenol and

triclosan possessed antimicrobial activity against the majority of the organisms tested in planktonic growth. A greatly reduced antimicrobial activity was, however, noted when used to combat biofilms. The exception to this was eugenol, which retained much of its activity against biofilms cultured in MHB. Recently, eugenol's antibacterial activity against *P. mirabilis* was highlighted by Devi *et al*. (2013), who demonstrated that eugenol altered the cell membrane integrity of *P. mirabilis* (27). Our data also strengthens previous research regarding TTO activity, in particular terpinen-4-ol, as the single active constituent of TTO, and its activity *in vivo* (9, 28, 29).

Whilst in our study, biofilms were more tolerant to these natural agents compared with planktonic cells, sufficient antimicrobial effects were observed to warrant further investigation into the clinical potential of these agents. These observations should encourage clinical studies to examine the effect of washout solutions on the blockage of long term catheterised patients. An alternative approach is the incorporation of these agents into biomaterials used in catheter development, thereby generating a catheter surface which could inhibit the growth and swarming of *P. mirabilis*. There is the potential advantage to using such agents prophylactically compared to antibiotics (*i.e* as they would not encourage the development of antibiotic resistant organisms).

In conclusion, this study suggests that triclosan, terpinen-4-ol and eugenol inhibit growth and swarming of *P. mirabilis* and may prove clinically useful for the treatment of CAUTIs. However, more work is needed to validate the biocides for washout solutions or their incorporation into urinary catheters.

ACKNOWLEDGEMENTS:

The authors acknowledge the financial support provided to this research by the Welsh Assembly Government under the Academic Expertise for Business (A4B) Collaborative Industrial Research Project (CIRP) scheme. The advice and support provided to us by our industrial partners Great Bear Healthcare, Cardiff, UK, and MBI Wales Ltd, Newport, UK, is also acknowledged.

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