1 **Biocide activity against urinary catheter pathogens (51 characters with**

2 **spaces-not to exceed 54 characters)**

3 **Biocides against urinary pathogens (31 characters)**

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24 **Abstract**

25 Antimicrobial effects of essential oils against bacteria associated with urinary

26 catheter infection was assessed. Tests were performed on 14 different bacterial

27 species cultured either planktonically or as biofilms. Biofilms were found to be up to

28 8-fold more tolerant of the test agents. Higher antimicrobial tolerance was also

29 evident in tests conducted in artificial urine. Eugenol exhibited highest antimicrobial

30 effects against both planktonic cells and biofilms when compared with terpinen, tea

31 tree oil and cineole.

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33 (73 words, cannot exceed 75 words)

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35   Foley catheters are frequently used to drain urine from the bladder of patients with

36   urinary incontinence or neurological dysfunction. Whilst providing a valuable

37   function, urinary catheters also provide access for microorganisms to infect the

38   bladder and also undermine the basic host defences of the urinary tract. Importantly,

39   catheter-associated urinary tract infections (CAUTIs) represent the most frequently

40   encountered hospital acquired infection (1). *Proteus mirabilis* is a urease positive

41   bacterium that raises urine pH during infection. This allows struvite and apatite

42   crystal encrustation of the catheter which obstructs urine flow, potentially promoting

43   serious clinical complications. All currently available catheters are vulnerable to this

44   encrustation and there is no single effective preventative strategy (2-4).

45   Due to the increasing prevalence of antibiotic resistance, interest has arisen in the

46   therapeutic use of alternative medicines in combating infection (5-9). Naturally-

47   occurring biocides are effective in inactivating a wide variety of microorganisms as

48   they often target multiple bacterial sites and as such, are less prone to development

49   of resistance compared with antibiotics (10, 11). One possible approach to prevent

50   catheter encrustation would be to incorporate these biocides into catheter wash-out

51   solutions, into the catheter material itself or use the catheter retention balloon as a

52   reservoir for the delivery of the antimicrobial agent into the catheterised bladder (12-

53   16). Little is currently known about the susceptibility of *Proteus* to natural

54   antimicrobial agents hence the aim of this study was to examine antimicrobial activity

55   of several essential oils, namely tea tree oil, terpinen, cineole, and eugenol, against

56   *P. mirabilis* and other urease producing bacteria involved in CAUTIs. Activity of these

57   agents was tested against both planktonic cells and biofilms, as the latter frequently

58   exhibits enhanced resistance to traditional antimicrobials.

59 The isolates used in this study are presented in Table 1. The antimicrobial agents

60 tested were cineole, tea tree oil (TTO), terpinen, and eugenol. Overnight cultures of

61 test isolates were prepared in Mueller Hinton Broth (MHB) (17) or artificial urine

62 adjusted to pH 6.1, and containing 1% Tryptone Soya Broth (TSB)(18). Cultures

63 were adjusted to a 0.5 MacFarland standard (approximately 10^8 cells ml^{-1}) and

64 diluted 100-fold in MHB or artificial urine. Serial dilutions of the antimicrobial agents

65 were prepared in MHB or artificial urine supplemented with 0.002% (v/v) Tween 80

66 (Sigma Aldrich, UK). A 100-µl volume of each dilution of antimicrobial agent was

67 added to an equal volume of microbial suspension, giving antimicrobial

68 concentrations ranging from 0.008 to 8% (v/v). Controls included bacterial

69 suspensions containing no antimicrobial agent and uninoculated culture media. The

70 bacteria and antimicrobial agent were co-incubated aerobically in 96-well microtitre

71 plates for 24 h at 37°C. Microbial growth was determined by spectrophotometric

72 analysis (620_{nm}). Absorbance readings were standardised against 'microbial-free'

73 antimicrobial agent controls. The minimal inhibitory concentration (MIC) was defined

74 as the lowest concentration of antimicrobial agent which resulted in ≥80% reduction

75 in absorbance compared to the antimicrobial-free controls (19).

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76 A biofilm susceptibility test was also performed using the isolates described in Table

77 1. Isolates were incubated as described above, but without agitation to allow for

78 biofilm formation. Culture medium was removed and the biofilms were washed with

79 100-µl phosphate buffered saline (PBS) to remove planktonic cells. Fresh culture

80 medium (100 µl), containing antimicrobial agent at concentrations ranging from 0.008

81 to 8% (v/v), was added to each well. Controls, as previously described, were also

82 included. Biofilms were incubated in the presence of antimicrobial agent for 24 h

83 without agitation under the conditions described above before the supernatant was

84 removed and the biofilm washed ($\times$1) with PBS. Fresh culture medium (100 µl),

85 which did not contain antimicrobial agent, was added to the biofilms which were

86 disrupted by repeated pipetting. The turbidity (620_{nm}) of the re-suspended biofilm was

87 measured and again after incubation at 37°C for an additional 6 h and 24 h. The

88 relative growth of microorganisms was determined by the change in absorbance and

89 antibiofilm activity recorded as the lowest concentration of agent that demonstrated a

90 $\geq$80% reduction in absorbance compared to the control. All experiments were

91 performed in triplicate on 3 separate occasions in MHB and artificial urine.

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93 A summary of the results is presented in Table 1. Cineole and TTO had the lowest

94 antimicrobial activity against bacteria grown in MHB and were therefore not tested in

95 artificial urine. Highest antimicrobial activity by the essential oils against planktonic

96 growth in MHB occurred with eugenol and terpinen. However, when tested in artificial

97 urine, the activity of terpinen was noticeably reduced. There was also a higher

98 tolerance of biofilms (up to 8-fold) to most of the essential oils compared with their

99 planktonic equivalents. This increased biofilm resistance was least evident with

5

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

101   artificial urine.

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103   Urinary catheters provide a convenient means to drain urine from the bladder of

104   patients suffering from urinary incontinence or neurological dysfunction. However,

105   they are also associated with complications, as they provide access for bacteria from

106   a heavily contaminated external skin site to the bladder and kidneys (20). Catheters

107   also undermine the normal filling and emptying of the bladder which flushes out

108   microorganisms that might be contaminating the urethra. Furthermore, a reservoir of

109   residual urine remains in the bladder of catheterised patients, allowing continued

110   proliferation of contaminating organisms (21). CAUTIs are usually asymptomatic,

111   and because of the danger of promoting antibiotic resistance, catheter associated

112   bacteriuria is generally not treated with antibiotics (22-24). Elimination of *P. mirabilis*

113   by appropriate therapy as soon as it enters the catheterised urinary tract would

114   reduce the incidence of CAUTIs and improve the quality of life for many patients,

115   whilst also reducing the costs of managing the complications of catheter encrustation

116   and blockage (25). Several management and treatment strategies for CAUTIs have

117   been used, including limiting catheter use, removal of the catheter as soon as

118   possible, maintaining a closed drainage system, and use of alternative catheter

119   surfaces with anti-infective agents (23, 26). Unfortunately, no single effective strategy

120   for prevention of CAUTIs has yet been identified.

121   The purpose of this study was to assess the antimicrobial activity of several essential

122   oils against bacteria involved in CAUTIs. Based on these findings, further studies are

123   planned to incorporate these agents into urinary catheter materials to prevent

124   infection. The results of this study show that TTO, terpinen-4-ol, eugenol and

125 triclosan possessed antimicrobial activity against the majority of the organisms

126 tested in planktonic growth. A greatly reduced antimicrobial activity was, however,

127 noted when used to combat biofilms. The exception to this was eugenol, which

128 retained much of its activity against biofilms cultured in MHB. Recently, eugenol's

129 antibacterial activity against *P. mirabilis* was highlighted by Devi *et al*. (2013), who

130 demonstrated that eugenol altered the cell membrane integrity of *P. mirabilis* (27).

131 Our data also strengthens previous research regarding TTO activity, in particular

132 terpinen-4-ol, as the single active constituent of TTO, and its activity *in vivo* (9, 28,

133 29).

134 Whilst in our study, biofilms were more tolerant to these natural agents compared

135 with planktonic cells, sufficient antimicrobial effects were observed to warrant further

136 investigation into the clinical potential of these agents. These observations should

137 encourage clinical studies to examine the effect of washout solutions on the

138 blockage of long term catheterised patients. An alternative approach is the

139 incorporation of these agents into biomaterials used in catheter development,

140 thereby generating a catheter surface which could inhibit the growth and swarming of

141 *P. mirabilis*. There is the potential advantage to using such agents prophylactically

142 compared to antibiotics (*i.e* as they would not encourage the development of

143 antibiotic resistant organisms).

144 In conclusion, this study suggests that triclosan, terpinen-4-ol and eugenol inhibit

145 growth and swarming of *P. mirabilis* and may prove clinically useful for the treatment

146 of CAUTIs. However, more work is needed to validate the biocides for washout

147 solutions or their incorporation into urinary catheters.

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