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Development of a broth microdilution method for biocide susceptibility testing of bacterial isolates using four reference strains

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Background and objectives: In contrast to biocide efficacy testing, biocide susceptibility testing (BST) lacks standardized methods for monitoring pathogens in human and veterinary medicine.
Materials and methods: The reference strains Staphylococcus aureus ATCC® 6538, Enterococcus hirae ATCC® 10541, Escherichia coli ATCC® 10536 and Pseudomonas aeruginosa ATCC® 15442 were investigated seven times by broth microdilution for their minimal inhibitory concentrations (MICs) towards benzalkonium chloride, glutardialdehyde, chlorhexidine and isopropanol. All tests were performed using tryptic soy broth as test medium. The following parameters were tested: i) 1st subculture (SC), and 2nd SC, ii) inoculum preparation by direct colony suspension (DCS) with/without glass beads (GB), iii) inoculum density according to the German Veterinary Association (DVG) or the Clinical and Laboratory Standards Institute (CLSI), and iv) incubation at 37°C for 24, 48, and 72 h.
Results: Increased incubation times resulted in higher MIC values. Comparing the results for the different times revealed that the highest stability of the values was seen after 24 h. Therefore, the following proposal is made: use of a fresh overnight culture (1st SC or 2nd SC), inoculum preparation via DCS with or without GB, inoculum density according to CLSI or DVG, and incubation at 37°C for 24 h.
Conclusion: This method can contribute to the harmonization of BST of bacterial pathogens in routine diagnostics.