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Abstract (publication only)

Difference in biofilm-forming abilities among faecal, urine and blood enterococcal isolates of human origin

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Objectives: The present study investigated the biofilm forming abilities of 60 *Enterococcus faecium* isolates recovered from blood, fecal and urine samples of hospitalized patients. **Methods:** To quantify biofilm production of 60 *E. faecium* strains (27 fecal, 21 urine and 12 blood isolates), overnight cultures were diluted 1:40 in Trypticase soy broth (TSB) supplemented with 0.25 % and 1 % glucose. A 200 µL sample of the cell suspension were placed, in triplicate, into 96-well polystyrene microtiter plate. After incubation at 37 °C for 24 h or 48 h, the wells were rinsed gently with 200 µL of PBS (pH 7.5) three times, biofilm samples dried at 60 °C for 1 h and fixed with methanol (95 %, v/v). Following, the methanol was removed and formed biofilms were stained with crystal violet (1% aqueous solution) for 20 min. The wells were subsequently washed with sterile PBS to wash off the excess crystal violet. The crystal violet-stained biofilm was made soluble by use of ethanol:acetone (80:20 v/v) and the absorbance of the extracted crystal violet was measured at 595 nm using microplate reader. All biofilm assays were performed in triplicate. **Results:** Human *E. faecium* isolates from both blood and fecal samples produced 66.6 % and 55.5 % biofilm, respectively in TSB with 0.25 % glucose supplementation and 24 h incubation. Under same circumstances 28.5 % of urine isolates formed a strong biofilm. At glucose concentration of 0.25 % and 48 h incubation, biofilm production by urine samples increased to 80.9 %. However, dramatic fall in numbers of biofilm producers among blood (8.3 %) and fecal samples (11.1 %) was determined. On the other side with the increase of glucose concentration in TSB from 0.25 % to 1 %, the number of strains able to produce biofilm among urine isolates increased to 85.7 %, following the 24 h incubation. Despite that by the increase of glucose concentration to 1%, number of strains able to produce biofilm among blood and rectal isolates decreased to 25 % and 40.7 %, respectively. The optimum conditions for biofilm production for all isolates was determined as TSB with 1 % glucose and 48 h incubation since 90.5 %, 58.3 % and 96.3% of urine, blood and rectal isolates produced biofilm, respectively. **Conclusion:** *Enterococcus faecium* isolates from fecal and urine samples produced more biofilm at optimal conditions than blood isolates. Urine samples exhibited different biofilm producing characteristics from blood and fecal isolates.