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THE RESEARCH OF MICROBIOLOGICAL STABILITY IN ANTI-DOPING SAMPLES

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INTRODUCTION

Urine samples collected for anti-doping analysis are at high risk of bacteria contamination, that may affect their chemical composition. When all steps of the procedure (cooling, storing and transporting) are taken properly, contamination is kept at a very low level, and it is not a significant problem.

However, when any part of this procedure is neglected, bacteria can multiply and in this case their influence can't be omitted.

AIM OF THE STUDY

The aim of this experiment was to study the possible impact of selected bacterial strains on the results of anti-doping analysis.

MATERIALS AND METHODS

In this research samples of pooled urine from 5 healthy men were used. The samples were adjusted to pH 7 by a few drops of concentrated NaOH solution. In this experiment the concentrations of endogenous androgens - naturally found in human urine – were determined. Standards of metabolites of nandrolone and THC to monitor the effect on some exogenous substances.

The urine used wasn't sterile. It was contaminated by following bacteria: *Enterococcus faecali* – in the number of 3700 CFU/ml and *Stenotrophomonas maltophilia* – 1300 CFU/ml.

For this study the following bacterial strains were selected: *Candida albicans, Escherichia coli, Enterococcus faecalis, Pseudomonas aeruginosa, Staphylococcus aureus,*

Stenotrophomonas maltophilia, Staphylococcus epidermidis, Proteus vulgaris. Last two strains were selected only because their ability to cause pH changes, and were not incubated separately.

The urine was divided into samples of 8.5 ml, and placed in sterilized test tubes. The samples were divided in groups of six. Each one was spiked with 50 µl of chosen bacteria suspension, containing from $1{,}5x10^5$ to $2{,}5x10^7$ CFU. Concentration of these suspensions was evaluated using the serial dilution method. The dilutes 10^{-6}, 10^{-7} and 10^{-8} were incubated for 24h on special culture media (agar for bacteria, and Sabouraud for *C. albicans*) and after that the formed cultures were counted.

Three samples to which the bacteria were not added were frozen in temperature of -70°C. They were treated as zero point. Spiked material was incubated in temperature of 35°C in a dark place. Half of the samples were incubated for 1 week and the rest for 2 weeks. Simultaneously a group containing only bacteria found in the original material was incubated in the same conditions. It was treated as the experiment's control group.

After the incubation period the samples were frozen in temperature of -70°C and then analysed according to the screening procedure for anabolic agents (screening 4 based on procedure described by Geyer et al (1998) [2]; the glucuronides and the free fraction were analyzed together – without separation to analyse free fraction of testosterone and/or epitestosterone) on GC/MS.

RESULTS

Obtained results are shown in Fig. 1-4 in comparison to zero and control group. The most significant changes in the steroid profile were caused by *E. faecalis* and *E. coli*. The stability of metabolites of nandrolone and THC was most influenced by - *E. Faecalis, E. Coli, P. aeruginosa, S. aureus, S. maltophilia and C. albicans*. In all samples, with significant T/Et ratio changes, it was caused by lowered testosterone concentration. Also 5α- and 5β-androstane-3,17-dione was observed, but its concentration was not measured.

CONCLUSION

False positive results (according to doping of testosterone) caused by bacterial contamination were not found, but false negative results for testosterone in principle are possible.

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Fig 1 - Changes in the testosterone/epitestosterone ratio.

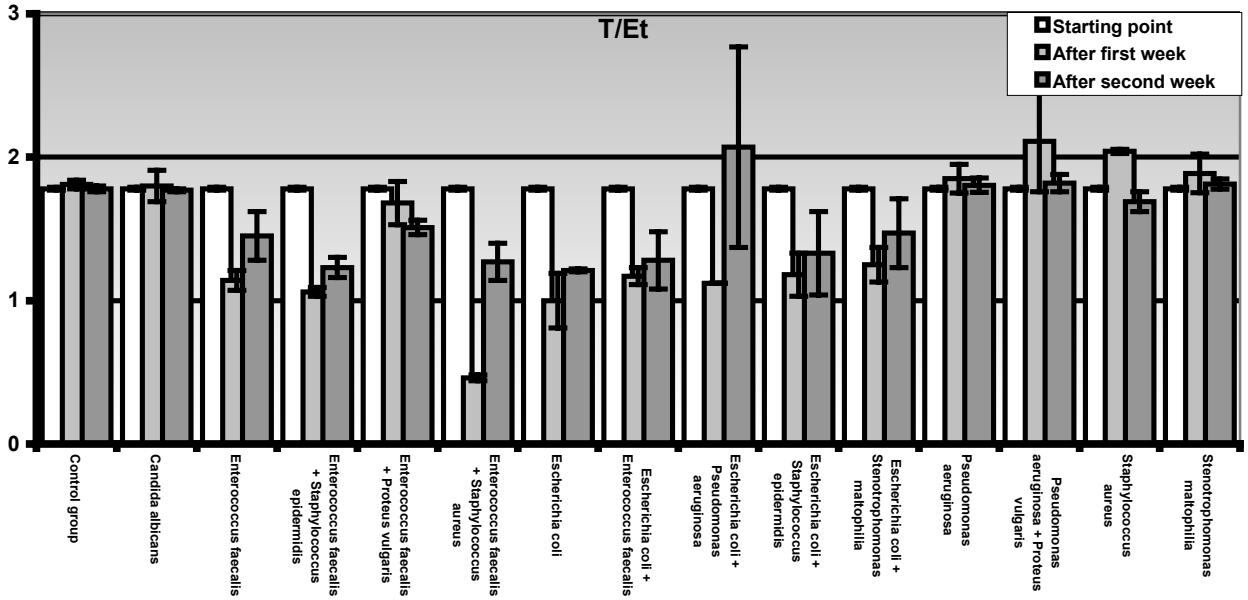

Fig 2 - Changes in the androsterone/etiocholanolone ratio.

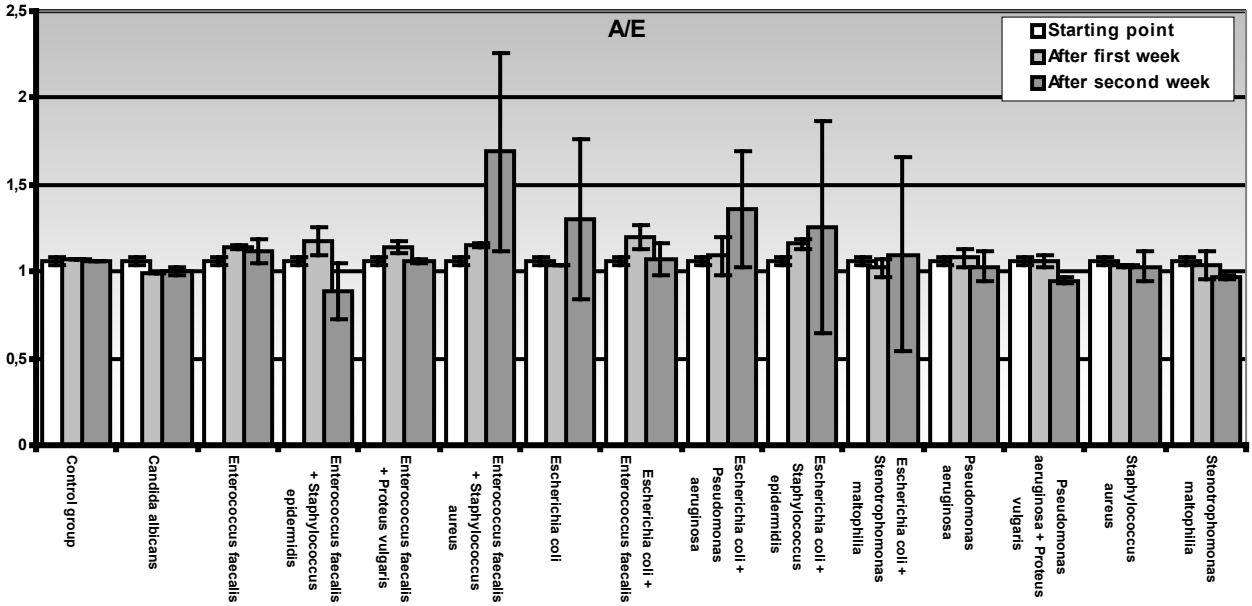

Fig 3 - Changes of concentration of 9-COOH-THC.

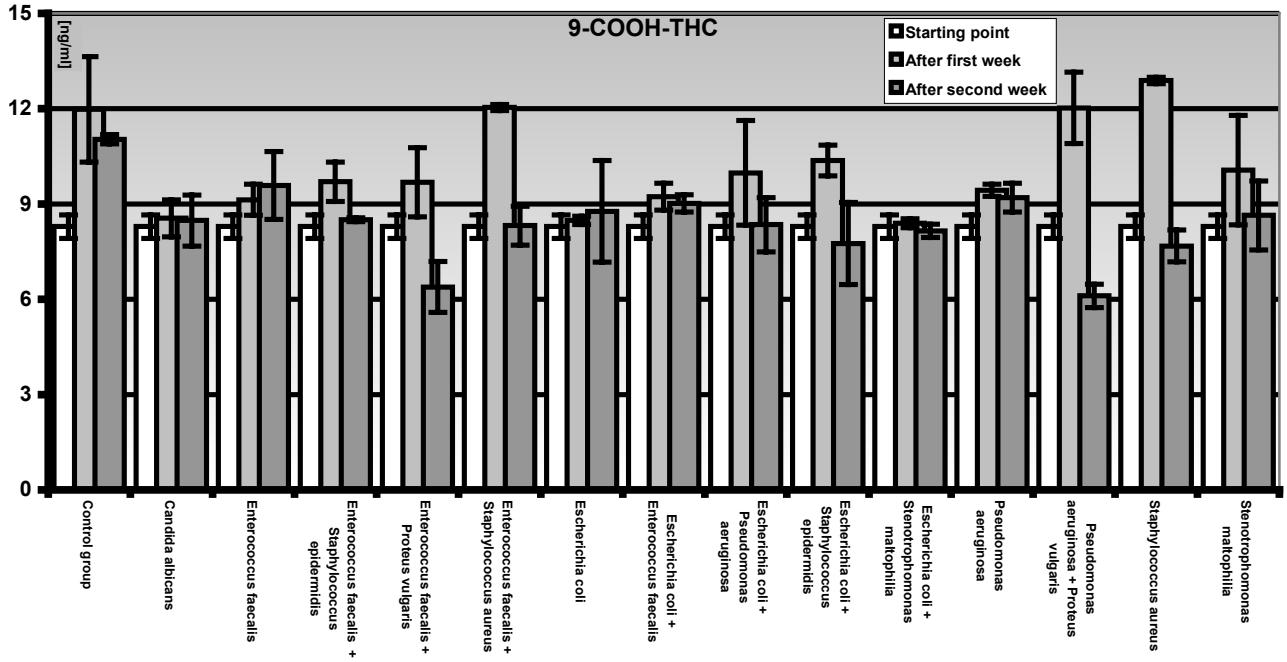

Fig 4 - Changes of concentration of 19-norandrosterone.