JAGIELLONIAN CENTER OF INNOVATION

Report of data analysis

Microbiological test of cosmetic files

Report for

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POLSKI ZWIĄZEK PRZEMYSŁU KOSMETYCZNEGO

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1. Purpose of the analysis

The purpose of analysis was to determine the number of bacteria and fungi and to identify microorganisms from cosmetic files: those used for cosmetic treatment and files disinfected with a broad-spectrum agent 2-3 days after the cosmetic treatment and 14 days after the cosmetic treatment.

The study also visualized the fungus growth on the file used for the cosmetic treatment.

Description of the tested samples and time intervals

Table 1 presents information on the samples provided for microbiological testing.

Table 1. Description of the set of cosmetic files used in the research.

Set		Date of the test
I	a) Disinfected file b) The file used for the treatment	2-3 days after cosmetic treatment
II	a) Disinfected file b) The file used for the treatment	14 days after cosmetic treatment
III	a) The file used for the treatment	Observation of fungal growth after 5 and 10 days of incubation

2. Test conditions

Table 2 presents information on the materials used for the test and the test conditions.

Table 2. Description of materials and conditions for the test.

Microbiological media	TSA agar – for total number of microorganisms Sabouraud's agar– for total number of fungi
Incubation temperature	30°C ± 1°C – for bacteria 25°C ± 1°C – for fungi
Incubation time	72h ± 2h – for bacteria 5 days – for fungi

3. Research method

3.1 Quantification of microorganisms

The quantification of bacteria and fungi was performed using the Koch decimal dilution method. The cosmetic files was suspended in 100 ml of buffered peptone water and mixed thoroughly. Serial dilutions from 10^{-1} to 10^{-5} were then made and 100 µl of each dilution was plated on the individual culture media. The plates were incubated aerobically. After the incubation time had ended, the colonies were counted.

3.2 Identification of microorganisms

After the incubation time was ended, the grown bacterial colonies were collected for identification using a MALDI-TOF mass spectrometer. The method of direct transfer was used to carry out the identification. In turn, the grown colonies of filamentous fungi were multiplied in Sabouraud's broth and after 48 hours of incubation, identification was made by extraction.

The results of MS MALDI-TOF identification were included as appendices to the report (Appendices: 220527-MB-INTER 372, 374 and 420). In the reports, under the results of the analysis, there is a description that identifies the tested sample.

3.3 Visualization of fungal growth on a cosmetic file

The cosmetic file used for the treatment was placed on Sabouraud's culture medium. The prepared sample was placed in an incubation at 25 ° C ± 1 °C. The fungus growth on the file was observed every few days.

Below are photos of the cosmetic file used for the treatment after 5 days of incubation and 10 days of incubation.

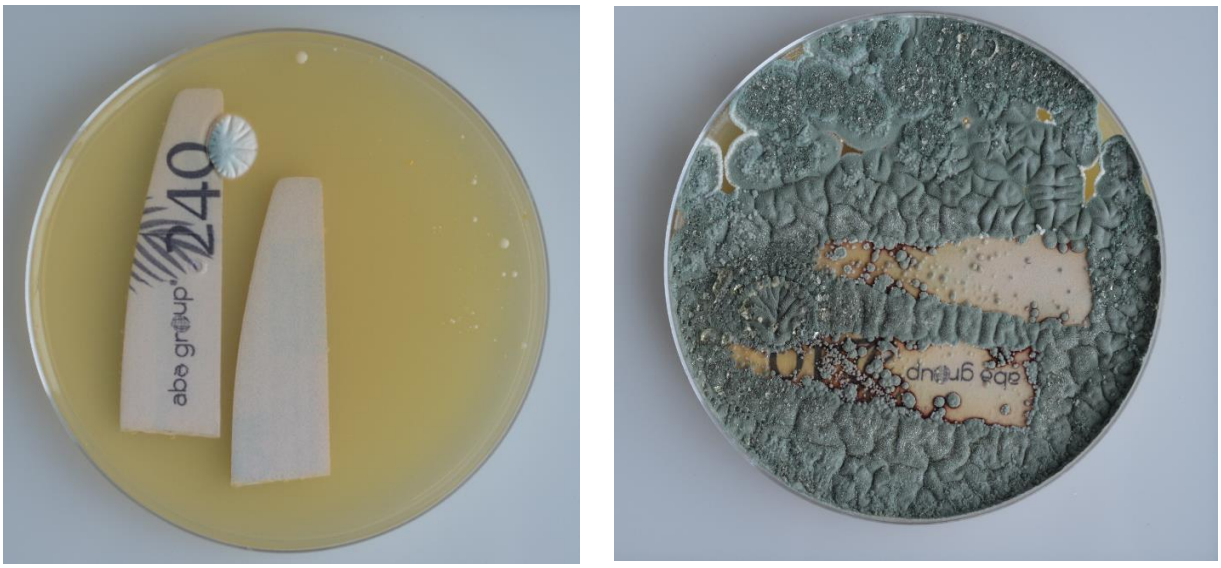

Figure 1. Figure 1 Growth of filamentous fungi on the file used for the treatment after 5 days of incubation (left) and 10 days of incubation (right)

4. Results

Tables 3 and 4 shows the results for the total number of microorganisms and the number of fungi. The results are expressed in the unit CFU - colony forming unit.

Table 3. Total Microbial Counts and Fungi Counts for Set I (2-3 days post treatment)

Set I	Total number of microorganisms [CFU]	Number of fungi [CFU]
The file used for the treatment	$6{,}0x10^2$	$4{,}0x10^1$
Disinfected file	$2{,}1x10^2$	$3{,}0x10^1$

Table 4. Total Microbial Counts and Fungi Counts for Set II (14 days post treatment)

Set II	Total number of microorganisms [CFU]	Number of fungi [CFU]
The file used for the treatment	$2{,}9x10^2$	$2{,}x10^1$
Disinfected file	$<1{,}0x10^1$	$<1{,}0x10^1$

5. Summary

The aim of the laboratory test was to check the growth of fungi and bacteria on cosmetic files after their use in a cosmetic procedure and after disinfecting them with a broad-spectrum agent. On the basis of the quantification studies carried out, no significant differences were found in the number of microorganisms and

fungi, regardless of whether the file was subjected to disinfection and regardless of the storage time of the file from the day of the cosmetic procedure (the same order of magnitude).

The study also performed the identification of the grown microorganisms using a MALDI TOF mass spectrometer. In the case of the file used for the treatment, but not subjected to disinfection, the presence of characteristic pathogens inhabiting the human epidermis, e.g. cocci from the genus *Staphylococcus* (*S. borealis* and *S. hominis*) and *Micrococcus luteus* [1]. The presence of *Rothia koreensis* has also been detected, which may be dangerous for immunocompromised people. Filamentous fungi such as *Penicillium expansum* [2] and *Penicillium brevicompactum* [3], which produce dangerous mycotoxins, were identified on the used cosmetic files. In the case of cosmetic files that have been disinfected, the presence of bacteria and filamentous fungi was also found. Filamentous fungi of the genus *Penicillium* were found in a visualization study showing the growth of microorganisms on the file after use in a cosmetic procedure. After 10 days, intensive fungal growth was observed due to the spreading of their spores.

Bibliography

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End of test report