MORPHOLOGICAL AND BIOCHEMICAL FEATURES OF MICROORGANISMS INHABITING THE SURFACES OF PERSONALIZED FEMORAL IMPLANTS

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Introduction
Estimation and characterization of bioburden on healthcare products is a crucial step in the determination of sterilization parameters [1]. Evaluation of this parameter is also necessary to set up the contamination control program for a sterilization procedure [2]. A thorough knowledge of the physiological and biochemical characteristics of the microorganisms, colonizing the surfaces of medical implants, allows adjusting the method and dose of the sterilizing agent [3]. The study presents the results of the assessment of the biodiversity of microorganisms, colonizing the prototypes of femur implants, immediately after their manufacturing.

Materials and Methods
Five femoral implants were used in the research. Before the analysis, the implants were cleaned from post-production contamination. The number of facultative non-fastidious, aerobic, spore-forming and anaerobic bacteria, as well as yeasts and moulds, were determined by membrane filtration, according to PN-EN: ISO 11727-1 and Polish Pharmacopoeia, edition X. The morphology of isolated bacteria was observed under the light microscope (BX63, Olympus, Tokyo, Japan) after Gram staining. The biochemical features were identified using Api®ZYM tests (BioMerieux, Marcy-l'Étoile, France), as well as Bactident® Oxidase (Merck, Darmstadt, Germany) and Bactident® Aminopeptidase (Merck, Darmstadt, Germany) following the manufacturer's instructions.

Results and Discussion
The surfaces of the implants were contaminated by bacterial strains. Yeast and moulds were not detected (TABLE 1).

TABLE 1 Determination of bioburden

№	Number of facultative, non-fastidious, aerobic bacteria	Number of anaerobic bacteria	Number of spore-forming bacteria	Number of yeasts and moulds
	cfu/implant			
1	nd	2	1	nd
2	4	nd	nd	nd
3	2	nd	nd	nd
4	nd	1	nd	nd
5	nd	nd	nd	nd

nd – not detected

Facultative and aerobic bacteria were the dominant microflora. However, anaerobic and spore-forming bacteria were also present. Their morphologies were described based on microscopic images (FIG. 1).

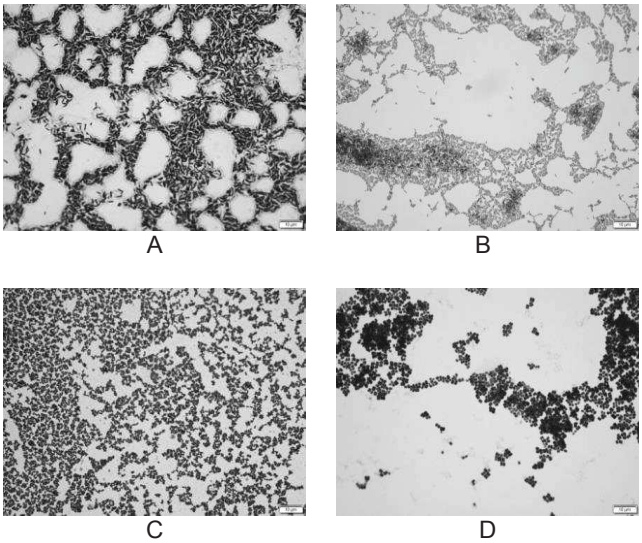

FIG. 1 An exemplary morphology of isolated bacteria, Gram-positive bacilli (A), Gram-negative rod shaped (B), Gram-positive cocci (C), Gram-negative cocci (D)

Most of the isolated strains were Gram-positive, cocci. We also identified Gram-positive bacilli, Gram-negative cocci and Gram-negative rod-shaped bacteria. Despite the morphological similarities of the Gram-positive cocci, the isolated strains showed slightly different biochemical characteristics. The differences concerned mainly alkaline phosphatase, leucine arylamidase and β-glucosidase. Large variations of enzymatic activities were also demonstrated by Gram-positive bacilli. Inequalities were related to oxidase, valine arylamidase, α-galactosidase, β-galactosidase and β-glucosidase.

Conclusions
The bacteria were dominant microflora colonizing the surfaces of the implants. Although the number of tested microorganisms was relatively small, the biochemical and morphological characteristics showed significant diversity. A cursory analysis of bioburden can increase the risk of incorrect adjustment of sterilization conditions and thus, the risk of patient infection.

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